AD NUMBER

ADB242926

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info; Oct 98 Other requests shall be referred to USAMRMC, Ft. Detrick, MD 21702-5012

AUTHORITY

USAMRMC ltr, 23 Aug 2001

AD_____

CONTRACT NUMBER DAMD17-96-C-6100

TITLE: Conditioning Military Women for Optimal Performance: Effects of Contraceptive Use

PRINCIPAL INVESTIGATOR: Lawrence E. Armstrong, Ph.D.
Carl M. Maresh, Ph.D.

CONTRACTING ORGANIZATION: University of Connecticut
Storrs, Connecticut 06269-1110

REPORT DATE: October 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-96-C-6100
Organization: University of Connecticut
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Patricia C. Modern 3/30/99

_____ _____

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1998	Annual (23 Sep 97 - 22 Sep 98)

4. TITLE AND SUBTITLE
Conditioning Military Women for Optimal Performance: Effects of Contraceptive Use

5. FUNDING NUMBERS
DAMD17-96-C-6100

6. AUTHOR(S)
Lawrence E. Armstrong, Ph.D.
Carl M. Maresh, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Connecticut
Storrs, Connecticut 06269-1110

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Oct 98). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The purpose of this investigation is to discover the effects of a physical training plus heat acclimation program on exercise performance, thermoregulation, immune function, and reproductive and stress hormone responses in three groups of women: oral contraceptive (OC), Depo-Provera (DEPO) contraceptive, and eumenorrheic-ovulatory (EU-OV) no contraceptive. The two-year total number of subjects was 22 (EU-OV, 9; ORAL, 10; DEPO, 3). DEPO was not statistically compared to the EU-OV and ORAL groups, due to the small number of subjects, but means $\pm$ SE are reported. All subjects were stronger, more physically fit, leaner, and heat acclimated at the end of the 8-week physical training program. In terms of reproductive hormones, study participants responded to the training regimen unremarkably. The ovulatory status of the eumenorrheic group at post-training appears similar to the pre-training period. Cycle length, follicular phase length, and luteal length appear to have remained unchanged. In terms of aldosterone and the stress hormones, results also are unremarkable. Trends in the data suggest that oral contraceptives moderately activate both the humoral and cellular immune systems. This may mean that oral contraceptives improve the ability of female soldiers to resist infections. Year III observations will clarify this hypothesis.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Defense Women's Health Research Program oral contraceptives, Depo-Provera, physical training, heat acclimation, reproductive hormones, endotoxemia, immune system	41
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

__X__ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

__X__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Lawrence E. Armstrong 10/16/98

PI - Signature Date

Table of Contents

Introduction

BACKGROUND

Of the 340,000 women in the Armed Forces of the United States, two-thirds (200,000) serve on active duty and are between the ages of 18 and 30 years. Although they are healthy and active, the unique challenges that military women face (i.e., basic training, physical training, combat) far exceed those in civilian occupations. For example, deployment introduces many unique stressors including harsh environments, primitive housing/sanitary standards, exposure to novel diseases, and close quarters that may affect heat tolerance and immune function.

It is important that an encounter with simultaneous multiple stressors has been recognized as a prominent etiologic factor in military casualties (23,53). For example, concurrent multiple stressors such as a sudden increase in physical training, fever or disease, dehydration, and a lengthy heat exposure were reported during the 5 days prior to exertion heatstroke in 10 soldiers (5).

CONTRACEPTIVE USE BY MILITARY WOMEN

Because military women share quarters with men, stressors related to sexuality arise. These include lack of privacy, a four-to-one ratio of males to females, sexual harassment, and unplanned pregnancy (18). At any point in time, approximately 18,000 active-duty military women are pregnant (61). The majority of these pregnancies are unplanned, in women under the age of 25 (67 %) (25). The need for contraception in the U.S. Armed Forces is authentic, considering individual career advancement, financial resources and mission priorities.

It is difficult to determine accurate statistics regarding the number of military women who use contraceptives (personal communication, LTC Katy Reynolds M.D., Nov 1995). But, it is known that 56% of military women who experience unplanned pregnancy use some form of contraceptive (25). Medical publications (57,59) indicate that 95 % of all sexually active civilian women aged 15 - 44 years, and 74 % of sexually active college females, use some form of contraception in the United States.

A woman's choice of contraceptive method is affected not only by the perceived efficacy and convenience of the technique, but whether additional risks or benefits are associated with its use. While oral estrogen and progestin contraceptive therapies remain the most popular method of pregnancy prevention in the United States, little is known about their effects on exercise performance, thermoregulation, immune function, or reproductive physiology.

The use of long-acting contraceptive methods is increasing in the U.S. Armed Forces because they simplify compliance. For example, Depo-Provera (depot medroxyprogesterone acetate), a long-acting (3-6 months) injectable agent, has an extremely low failure rate (0.0-1.2 per 100 woman-years) and is used by 11 million women in over 90 countries, including the United States (22,58). The U.S. Food and Drug Administration approved its use in 1992, based on WHO epidemiologic data.

Depo-Provera typically provides a three month window of safe and effective contraception, and is ideal for use in military settings (i.e., basic training, deployment, combat). One injection provides a female soldier with three months of uncomplicated birth control. This contraceptive technique is worthy of study because it is used by an ever-increasing percentage of military women and can be administered safely, with little or no supervision, for many years. Although Depo-Provera is the most widely studied injectable steroid formulation (over 500 investigations involving its effectiveness and safety have been published since it became available 29 years ago), very little is known about its effects on exercise performance, thermoregulation, or immune function.

ORAL CONTRACEPTIVES, IMMUNE FUNCTION, AND RESPONSES TO EXERCISE-HEAT-DEHYDRATION

Thermal balance may be altered by phase of the menstrual cycle, probably due to increased progesterone levels during the luteal phase (40). For example, exercise during the **luteal** phase is characterized by a higher T_{core} (0.4°C) and a higher T_{re} sweat threshold temperature (0.25°C), versus the **follicular** phase (31,40). Although these minor effects have minimal military relevance, they become more important if ambient conditions are hot and humid, and if exercise is intense and prolonged. One study demonstrated, for example, that a 0.6°C T_{re} difference (luteal versus follicular phase) occurred when women exercised for 60 minutes at 60% VO_2max in a 22°C and 60 % rh environment (46). Had the ambient temperature been 35-40°C, the difference between luteal and follicular phase responses would probably have been greater. Admittedly, these minor effects are not as militarily relevant as the effects of oral contraceptives on thermal balance and exercise performance. Although little is understood, it

has been shown that oral contraceptives users exhibit more uniform T_{core} and sweating responses than non-users, probably because there is no phasic alteration of **progesterone** levels in these women. Further, injected progesterone (i.e., Depo-Provera) increases basal T_{core} within 24-36 hours of drug administration (31). The impact of these findings on heat tolerance may have significant implications for military women in basic training, deployment, or combat settings and we are examining these issues.

Most military stressors lead eventually to a common response pathway involving activation of the sympathetic nervous system, the secretion of cortisol and epinephrine. The hypothalamic response begins with secretion of corticotropin-releasing hormone (CRH). CRH stimulates secretion of **immunostimulants** (prolactin, LH, and FSH, TSH, growth hormone) and **immunosuppressors** (beta-endorphin, ACTH, cortisol, and alpha-melanocyte stimulating hormone). Plasma cortisol, which is elevated during stress, decreases the levels of antibodies and leukocytes, depresses the ability of white blood cells to digest phagocytized substances, and reduces fever. The degree to which these systems are activated depends on the total stress encountered, the previous physical and mental experiences of the individual, and the degree of control that she or he can exert over the stressful situation (32).

The mechanisms by which these contraceptives affect reproduction are described below (59).

Combined estrogen/progestin formulations: Both estrogen and progestin prevent ovulation by suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. This occurs via inhibition of hypothalamic gonadotropin-releasing hormone (GnRH) release. Levels of LH, FSH, progesterone, and estradiol are suppressed. Besides the inhibition of ovulation, cervical mucus composition is altered and ovum transport/implantation are modified (59).

Depo-Provera: A steroid that prevents follicular maturation, ovulation, and endometrial thickening by inhibiting the secretion of gonadotropins. Depo-Provera is similar in structure to naturally occurring progesterone. Contraceptive plasma levels of this compound are reached within 24 hours of injection and are sustained for 14 weeks after injection (38). Plasma levels of estradiol remain within the normal range (22,38).

Changes in the plasma levels of compounds relevant to reproduction and fertility may alter immune function in women. Elevated **estrogen** levels result in general immunosuppression (8,60) and decreased natural killer cell activity (8). High concentrations of estradiol also serve to suppress immune function in women (41). In contrast, low circulating estrogen is associated with increased total lymphocytes and CD_4^+ counts (34). Therefore, **oral contraceptives** (which contain both estrogen and progesterone) should affect the immune system differently from **Depo-Provera** (which acts similarly to progesterone) because various immune cells are affected differentially by estradiol and progesterone (41). Depo-Provera, theoretically, should enhance immune function, in comparison to oral contraceptives. It is not known, however, if such alterations are sufficient to cause illness, or if physical training and heat acclimation will affect specific components of the immune system. Species differences exist; rats and mice exhibit responses that are opposite to those described above for humans (1,42).

HEALTH & PERFORMANCE OF MILITARY WOMEN: RESISTANCE TO INFECTION

A robust immune system (i.e. a high titre of anti-LPS IgG, see below) is desirable for soldiers at all times. Conversely, the combination of overtraining and the stress of new surroundings suppresses immune function and often is blamed for illnesses (i.e. the common cold, sore throat, influenza, mononucleosis) that afflict soldiers and athletes during physical training (52).

The complexities and redundancies of the immune system (16), as well as the many differences in protocols of published studies (62), have contributed to a long-standing polarity of opinion regarding the influence of **acute exercise** and long-term **physical training** on immune function. Although it is known that both women and men show a marked leukocytosis (total white blood cells and polymorphonuclear neutrophils) following exercise of greater than 3 hours duration (63), few definitive conclusions are possible (16,55,62). Similarly, immunologic responses to other stressors inherent in military training (i.e., **dehydration, heat stress**) have not been investigated adequately in women. This is relevant to the present investigation because military training, especially for new inductees or field units that translocate to stressful environments, subject soldiers to **multiple stressors** in virtually all cases (37). These scenarios and multiple stressors may affect temperature regulation and other vital bodily processes negatively in women (24,45).

SOLDIER HEALTH AND PERFORMANCE: INTESTINAL VIGOR

In addition to immune function, the preceding scenarios illustrate the military relevance of normal nutrient delivery (especially water, salt, and carbohydrates) during physical training (7) and combat (3). However, the research of Gaffin and colleagues (12,15,27,28) and the review of Hubbard et al. (36) have revealed an immunological response to intestinal events, during concurrent multiple stressors, that is not widely appreciated.

During digestion, gram negative bacteria exist in chyme in the small and large intestines. Dead gram negative bacteria provide large amounts of the toxic cell wall component **lipopolysaccharide (LPS)**. LPS found in the outer membrane of gram-negative bacteria are known as **endotoxin**. High levels of plasma LPS seem to be the immediate cause of human septic shock (43). One of the most important discoveries in critical care medicine in the 1980s involved the recognition that LPS may leak out of damaged intestines into the blood, resulting in cardiovascular insufficiency, extensive organ damage or death, in severe cases.

THE PATHOGENESIS OF ENDOTOXEMIA

When LPS enters the portal circulation, one of three fates is possible: (a) detoxification by Kupffer's cells in the liver, (b) inactivation by binding to circulating factors (i.e., HDL, anti-LPS IgG, LPS binding protein, CD-14 or soluble CD-14 receptor), (c) expression of toxicity by binding to LPS binding protein (LBP) and subsequently to CD-14 receptors on the membranes of microphages and other cell types (28). This latter fate results in hypersecretion of **cytokines** (e.g., TNF, IL-1), toxic immune mediators that may cause fever, nausea, vomiting, diarrhea, headache, tissue injury, shock, or death. These symptoms are observed in many cases of heatstroke (35), and have led authorities to suggest that cytokine release is a risk factor for exertional heatstroke (36). Although heatstroke is unheralded and has an unknown etiology in most cases, autopsies of human heatstroke victims have found high titres of plasma LPS and cytokines (13,14). LPS also could be involved in heatstroke by suppressing sweating (9) or cardiac function (44).

Cytokines may alter soldier performance in other ways (23): (a) Both TNF and IL-1 can induce slow wave sleep, suppress appetite (39,54), and cause fever by stimulating prostaglandin E_2 synthesis (21). (b) TNF can induce all features of endotoxin-induced septic shock (43). (c) IL-1 changes the response of arteries to norepinephrine in different vascular beds, and may cause abnormal regional blood flow (47).

EXERCISE-HEAT STRESS, SPLANCHNIC ISCHEMIA, AND LPS

Compared to exercise in cool environments, exercise-heat stress produces a markedly reduced blood flow in splanchnic vascular beds concurrent with an increased heart rate (48). This diversion of blood flow contributes to increased skin blood flow (important for heat dissipation), but carries the threat of compromising the function of splanchnic organs (49,50). This is important because the removal of bacteria and other microorganisms is normally a function of the reticuloendothelial system (RES) in the liver (2). The splanchnic ischemia that accompanies sustained hyperthermia during exercise also has been proposed as a cause of heat exhaustion (6) and the intestinal illnesses seen in 20-30% of all marathon runners (11). If exercise-heat stress or ischemia is great, an increase in plasma LPS may occur due to increased gut permeability. This phenomenon has been observed in primates, cats, miniswine, and rats (28,51). These animals demonstrated that core **hyperthermia** must reach severe levels (42 - 45°C) before lethal increases in LPS occur (28,29,36).

Other stressors enhance the entry of gut-derived LPS into the circulation: hypovolemia, splanchnic artery occlusion, and diarrhea (28,36). Hypoxia also has been shown to potentiate the production of TNF and IL-1 in human blood mononuclear cells, after resting exposure to subthreshold levels of LPS (30).

Human studies suggest that **exercise and/or physical training** play an important role in endotoxemia. For example, plasma LPS levels were elevated after strenuous exercise by triathletes and ultramarathon (89.5 km) competitors: (a) Bosenberg et al. (12) found that LPS rose and the "natural" anti-LPS IgG (the antibody formed in response to LPS) decreased during competition; (b) Brock-Utne et al. (15) observed that 80 % of collapsed runners had elevated levels of plasma LPS. The casualties with low/normal levels of LPS, but high levels of anti-LPS IgG, symptoms were far less severe than those with high/abnormal plasma LPS, and low levels of anti-LPS IgG; this latter group required two days to recover. Thus, the presence of a higher titre of anti-LPS appeared to protect the runners, possibly because they had been autoimmunized during daily training.

A critical question has emerged from these human studies. Can the level of "natural" anti-LPS antibodies be manipulated in soldiers to effectively reduce susceptibility to heat illness? The hypothetical answer suggests that part of the benefit of **physical training** for soldiers might be to increase the natural plasma levels of anti-LPS

IgG. This could occur as small amounts of LPS enter the circulation, during strenuous training, on a daily basis. Because LPS stimulates a hypersecretion of the cytokines TNF and IL-1, this issue has great military relevance because cytokines may increase casualty rates (see above) and the susceptibility to heat illness (36).

Purpose

It is important that an encounter with simultaneous **multiple stressors** has been recognized as a prominent etiologic factor in military casualties (23,53). The goal of this study is to provide important information to reduce complications associated with stressful environments and therefore decrease casualties in military women. Comprehensive information about the health of military women facing multiple stressors is not currently available. Our study has been designed to clarify the ability of exercise training and heat acclimation to minimize the effects of multiple stressors on (a) exercise responses in the heat while dehydrated, (b) immunocompetence, and (c) hormone levels.

It is essential to the goals of this research project that we meticulously control the onset of testing and training for each subject's menstrual phase and status, and document compliance to contraceptive therapies.

Because the effects of oral and injectable contraceptives on physical training and heat acclimation are virtually unknown, and because the immune system maintains a constant state of personal health by interacting with every organ system in the body, the following technical objectives and hypotheses have great relevance to military women and military units.

Technical Objectives

A. Primary Longitudinal Objectives

1. To evaluate differences among the three groups with respect to immune function before and after an eight-week training/heat acclimation program.

2. To evaluate differences among the three groups with respect to the exercise-heat tolerance test (EHT) responses before and after an eight-week training/heat acclimation program.

3. To evaluate differences among the three groups with respect to reproductive hormone status before and after an eight-week training/heat acclimation program.

B. Secondary Longitudinal Objectives

1. To evaluate differences among the three groups with respect to stress hormones before and after an eight-week training/heat acclimation program.

2. To evaluate differences among the three groups with respect to body composition and VO_2max before and after an eight-week training/heat acclimation program.

C. Dependent Variables: Categorical Definitions

1. Reproductive hormone - estradiol, progesterone, sex hormone binding globulin

2. Immune function - CD-4+, CD-8+, anti-LPS IgG, total IgG, HSP_{70}, IL-10, IFNg
(abbreviations defined on page 13)

3. Exercise-heat tolerance -
   a) thermoregulatory markers: rectal temperature, skin temperature, whole body & local sweat rate, skin blood flow
   b) fluid-electrolyte balance: aldosterone, osmolality, hematocrit, hemoglobin, plasma volume shift
   c) exercise performance: heart rate, blood pressure, exercise tolerance time, rating of perceived exertion, rectal temperature, glucose, lactate

4. Stress hormones - cortisol, epinephrine, norepinephrine

D. Independent Variables:

A. groups: oral contraceptive users (ORAL), Depo Provera users (DP), eumenorrheic ovulatory women taking no form of birth control (EU-OV)

B. time: pre-training/heat acclimation
post-training/heat acclimation

Null Hypotheses

A. Null Hypotheses Associated with Primary Longitudinal Objectives

1. There will be no significant differences among the three groups with respect to immune function before and after an eight-week training/heat acclimation program. We expect that the Depo-Provera group will exhibit the most favorable immune response.

2. There will be no significant differences among the three groups with respect to the exercise-heat tolerance test (EHT) responses before and after an eight-week training/heat acclimation program. We expect that the Oral Contraceptive group will exhibit the most favorable thermoregulatory response.

3. There will be no significant differences among the three groups with respect to reproductive hormone status responses before and after an eight-week training/heat acclimation program. We expect that the EU-OV group will exhibit the greatest perturbations in reproductive hormone status.

B. Null Hypotheses Associated with Secondary Longitudinal Objectives

1. There will be no significant differences among the three groups with respect to stress hormones before and after an eight-week training/heat acclimation program. We expect that the magnitude of the changes in stress hormone levels will be equivalent among the groups.

2. There will be no significant differences among the three groups with respect to body composition and maximal aerobic power (VO_2max) before and after an eight-week training/heat acclimation program. We expect that the changes in body composition and VO_2max will be equivalent among the groups.

Statement of Work / Experimental Scope

Technical Objective: To evaluate the effects of oral and injectable contraceptives on hormones (i.e., reproductive, fluid-electrolyte, stress), immune system function, and exercise-heat-dehydration tolerance.

Task 1: Months 1 - 4: Order supplies, materials; prepare equipment and chamber. Oversee graduate students/ technicians and budgetary matters. Insure that research meets regulations of Environmental Health & Safety and the Institutional Review Board for Human Subjects.

Task 2: Months 4 - 7: Recruit, screen, identify, and brief test subjects. Conduct preliminary screening to eliminate subjects with exclusionary criteria.

Task 3: Month 8: Conduct Intensive screening of subjects. Select >15 subjects in three groups: oral contraceptive users, Depo-Provera users, and EU-OV. Collect descriptive subject data.

Task 4: Month 8: Collect two baseline blood measurements of immune system markers and hormones (reproductive, fluid-electrolyte), verify normalcy of reproductive function, and verify menstrual phase timing.

Task 5: Month 8: Prepare environmental chamber and instruments for testing. Conduct >15 exercise-heat tolerance tests (90 min each) at 38°C, to document pre-training responses.

Task 6: Months 8 - 10: Conduct eight-week training program for >15 women (6 days/week; 3 days involve heat exposure up to 90 min).

Task 7: Months 8 - 10: Collect blood samples throughout training period, to evaluate hormones (reproductive, fluid-electrolyte), immune system function, normalcy of reproductive function, and menstrual phase timing.

Task 8: Month 10: Prepare environmental chamber and instruments for testing. Conduct >15 exercise-heat tolerance tests (90 min each) at 38°C, to evaluate post-training responses. Collect blood samples to identify post-training levels of hormones, immune markers, and timing of menstrual phases.

Task 9:	Months 10 - 12: Laboratory analyses. Enter data into spread sheet. Submit annual report when required.
Tasks 10 - 17:	Months 13 - 24: Repeat Tasks 1 - 8 above, to begin collecting data on >15 additional women, bringing the number to 30 total subjects.
Tasks 16 - 25:	Months 25 - 36: Repeat Tasks 1 - 8 above, to begin collecting data on >15 additional women (goal: bring the number to 15 in each treatment group).
Task 26:	Submit final report when required.
Task 27:	Prepare abstracts for submission to scientific conferences. Prepare manuscripts for submission to journals for publication.

Experimental Methods and Procedures

This section presents the experimental design and procedures which have been used to meet study objectives. In order to achieve the necessary number of subjects (n = 15) for each group, we are performing all screening procedures, and training the three different groups, in multiple iterations over three years using a balanced distribution of subjects to control for variance.

SUBJECT CHARACTERISTICS

Female civilian students attending the University of Connecticut were recruited by announcements posted on bulletin boards, in classrooms, and the daily campus newspaper. The University of Connecticut has approximately 8,000 female students within the 18-34 year age range, and we are experienced in recruiting women for experimental and training studies. University staff and women living in adjacent communities also were recruited. Potential volunteers were given a description of the objectives, procedures, risks, and time commitments required for the study. All subjects were asked to provide written voluntary consent to participate, in compliance with the Institutional Review Board for Human Subjects at The University of Connecticut. Interested subjects completed a medical history and physical activity questionnaires, and were interviewed by one of the investigators.

All subjects are required to meet the following criteria:
a) aged 18 to 34 years; b) within the average range (± 2 SD) of U.S. military women for height (162 $\pm$ 13 cm) and weight (60 $\pm$ 16 kg); c) in good health, as determined by a medical and gynecological examination (private physician, within the previous 12 months) including a normal Papanicolaou smear; d) free of any chronic disease including thyroid disease and hyperprolactinemia; e) lack of any recent (within three months) changes in menstrual status; f) appropriate activity history; g) no history of eating disorder or depressive illness within the past three years and an appropriate score on the Eating Disorders Inventory (EDI); h) the absence of any contraindications revealed in a medical history that might preclude participation in the study, including a history of heat-related illness, endotoxemia, chronic respiratory disorder, cardiovascular disease, hypertension, metabolic disorders, convulsive disorders, drug or alcohol dependence; and i) not routinely taking a prescription or over-the-counter medication that would alter variables measured herein. All subjects must be non-pregnant, for the duration of screening and testing, as determined by blood sample analysis for HCGH. All University of Connecticut students are required to have current inoculations.

Subjects were asked to report any gastrointestinal or respiratory tract illnesses, or superficial injuries (i.e. abrasions, cuts) incurred during their involvement in the project. All subjects were performing no more than 90 minutes of aerobic activity per week for the previous 12 months, and had a maximal oxygen uptake ($V0_2$ max) less than 42 $ml \cdot min^{-1} \cdot kg^{-1}$. Subjects were paid for their participation.

INCLUSION OF MINORITY TEST SUBJECTS

Women representing minority groups have been encouraged to participate because nothing is known about racial differences in these responses. Their responses will be compared to non-minority subjects, in **post hoc** statistical analyses. The student body at the University of Connecticut includes 12 % minority representation. In fact, two graduate students in our doctoral program are women of African-American descent and will be involved in this project for its entire duration.

EXPERIMENTAL DESIGN

To test the various hypotheses set forth in this investigation, we are utilizing three groups of women: a) females currently ingesting an oral contraceptive (ORAL) for a minimum of three months prior to the study (n = 15), b) females receiving depot medroxyprogesterone acetate (DP; Depo-Provera) long-acting contraceptive therapy for a minimum of three months prior to the study (n = 15), and c) eumenorrheic ovulatory (EU-OV) females (n = 15).

During the Fall semester of academic year 1996-97, subjects were recruited and underwent a two month (during the months of October, November and December) preliminary screening procedure. Beginning in January, subjects performed an intensive one-month screening procedure, followed by an 8-week exercise training plus heat acclimation program. Exercise training sessions were held six days per week, with the heat acclimation sessions (alternate days, up to 90 min·day^{-1}) comprising three of those days. Maximal oxygen uptake tests and exercise-heat tolerance (EHT) tests were performed prior to, and at the end of the 8-week training program. It is our contention that a non-training control group is not necessary for this study for two reasons: a) because it represents a scenario with little or no military relevance and b) the pre- and post-training measurements for all groups in this study allow us to compare untrained and trained states.

Weight, menstrual patterns, nutritional habits, training habits, and any atypical stressors were monitored throughout the study. Subjects were weighed (kg) during all laboratory visits. Menstrual bleeding patterns and exercise reports were monitored daily via diary and training cards. Subjects recorded any medications that they were consuming in their menstrual diary. Seven day nutritional dietary records were completed during the first seven days of each menstrual cycle (or 28 day period) to make sure that dietary intakes are appropriate to support the nutritional demands and caloric expenditure of training. Thus, any significant changes in dietary habit were documented as thoroughly as possible. Each subject's health status is of great concern to us, from an ethical perspective, and to ensure that she will continue with the training program. The Women's Health Center on our campus is available for appointments and to collect clinical data concerning illness.

MENSTRUAL CYCLE AND COMPLIANCE MONITORING

All eumenorrheic ovulatory (EU-OV) subjects were asked to participate in menstrual screening procedures that accurately determine their ovulatory status and the length of their luteal phase. These were determined via blood samples that pinpoint the onset of the luteinizing hormone (LH) surge to within 12-24 hours. Subjects were asked to maintain menstrual logs to document menstrual cycle length and duration of menstrual flow days prospectively. During the menstrual cycle immediately preceding training, and during the second month of physical training, all eumenorrheic subjects had blood sampled during days 2, 3 or 4 (until 1 day after the peak LH concentration had been reached), one blood sample taken 7 days later, and one sample taken on day 23, to document menstrual phases. These blood samples were analyzed on the same day for estradiol (E_2), progesterone (P_4), luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG).

All subjects ingesting oral contraceptives (ORAL) were asked to report the exact preparation, duration of use and compliance to therapy. All oral preparations must be of the ethinyl estradiol type (over 25 commercial products exist). Women ingesting preparations that include mestranol were excluded from the study. Subjects were asked to provide empty pill packs to the investigators to document therapy information. During the cycle immediately preceding training, and during the two months of training, all ORAL users had blood sampled during day 2, 3, 4, or 5 following the onset of menses to document compliance to therapy. These blood samples were analyzed for E_2, P_4, and SHBG.

All subjects receiving long-acting Depo-Provera (DP) contraceptive therapy were asked to report the exact dose, preparation, and duration of use. During the cycle immediately preceding training, and during the two months of training, all injectable DP users had blood sampled during days 2, 3, 4, or 5 of a given 28 day period

(initiated on an arbitrary day) to document therapy. These blood samples were analyzed for medroxyprogesterone acetate (i.e., provided in the contraceptive DP), E_2, P_4, and SHBG.

BODY COMPOSITION AND MAXIMAL OXYGEN UPTAKE MEASUREMENTS

Body composition analyses were performed during the first seven days of each menstrual cycle or 28 day period, and at the beginning and end of the training period. Body density was determined from underwater weighing. Percent body fat and lean body mass were calculated according to Siri (56). All subjects completed an incremental run to exhaustion (modification of Costill and Fox protocol) on a motorized treadmill for determination of VO_2 max (17). These tests were performed during the intensive screening period and following the exercise training program. Briefly, subjects ran at an appropriate speed for four minutes at 0% grade. After four minutes, the grade was increased to 4% for two minutes. The grade was then be increased 2% every two minutes until the subject reached volitional exhaustion. Two of the three following criteria were used to verify the attainment of VO_2 max: 1) no further increase in VO_2 (less than 150 $ml \cdot min^{-1}$) with an increase in grade, 2) heart rate greater than 90% of predicted maximum (220 minus age), and 3) respiratory exchange ratio greater than 1.1.

EXERCISE-HEAT TOLERANCE TESTING

Exercise-heat tolerance (EHT) tests were performed at the beginning and end of the 8-week training program. To enhance the stress associated with the EHT, subjects undertook a 24-hour water restriction prior to testing, providing an approximate -3% level of dehydration. The EHT involved walking on a motorized treadmill at 93.6 $m \cdot min^{-1}$ and 5% grade (4). Walking speed was verified for each test with a hand-held tachometer (Model 8240-20 Cole Parmer Instrument Co., Chicago, IL). The mean temperature and % humidity were 38°C and 50-70%, respectively. Air flow was 2.3 $m \cdot s^{-1}$. No water was consumed during the EHT. The test was terminated if: a) T_{re} reached 39.5°C, b) the heart rate exceeded 180 beats min^{-1} for five consecutive minutes, c) the subject showed signs of heat illness, d) the subject asks to stop, or e) she completed 90 minutes of exercise.

A schematic representation of events during each EHT test appears as **Figure 1**. The following physiological and perceptual measures were taken at regular intervals before, during and after EHT testing: oxygen uptake, minute ventilation, and respiratory exchange ratio using an on-line system (Medical Graphics Corporation); whole-body sweat rate (± 50 g) via body weight differences; mean weighted skin temperature (4 sites) via infrared temperature scanner (Ototemp, Inc.); subjective ratings of perceived exertion (10); and thermal stress (64). Rectal temperature (rectal thermistor, YSI Inc.), heart rate via cardiotachometer (Polar Electro), and exercise time were the primary variables representing exercise-heat tolerance. Measurements of local chest sweat rate using resistance hygrometry (Model B1-102, Bi-Tronics, Inc.) and local skin blood flow via laser doppler flowmeter (Techtronics, Inc) will begin in Year II and continue through Year III (see section below titled, "Recommendations Regarding the Statement of Work" item 2).

EHT TESTING: MENSTRUAL PHASE AND CONTRACEPTIVE THERAPY

All eumenorrheic ovulatory women were tested during day 2, 3, 4, or 5 of their menstrual cycle (i.e., early follicular phase). All oral contraceptive users were tested on day 2, 3, 4, or 5 of the 7 day placebo period for their respective pill packs. All Depo-Provera users were tested on day 2, 3, 4, or 5 of a 28 day period arbitrarily initiated during the preliminary screening period. The specific day on which testing occurs remained consistent for each subject.

EXERCISE TRAINING PLUS HEAT ACCLIMATION

The exercise training program lasted 7-8 weeks--two menstrual cycles--in duration. It was necessary to admit subjects into the training program in a staggered fashion to account for timing differences in menstrual cycle phase and contraceptive therapy. Training sessions were held six days per week (Monday - Saturday). Training sessions on Tuesday, Thursday, and Saturday involved strenuous group running and calisthenics (push-ups and sit-ups), with a progressive increase in volume and speed of running across weeks. The number of push-ups and sit-ups also progressively increased for eight weeks. All of these training sessions were supervised. Training sessions on Monday, Wednesday, and Friday also were supervised, and involved exercise-heat exposures (environmental chamber, 36°C, 50-70 % rh) progressing toward 90 minutes of continuous exercise-heat acclimation each day. These sessions entailed 5-6 subjects exercising at one time, employing a circuit of bench stepping, cycle ergometry, and treadmill walking. Subjects were permitted to drink water ad libitum during these sessions. Subjects were

encouraged to exercise continuously as long as possible during these sessions, but were asked to remain in the chamber even if they stop exercising, for the complete 90 minute period. However, subjects were removed from the environmental chamber if: a) T_{re} reached 39.5°C, b) heart rate exceeded 180 beats·min^{-1} for 5 consecutive minutes, or c) the subject showed signs of heat illness.

RESTING BLOOD COLLECTIONS: HORMONE & IMMUNE SYSTEM ANALYSES

Figure 2 presents the timeline for resting hormone and immunological analyses during the preliminary screening, the intensive screening, and the 8-week heat acclimation/training program. Blood samples were obtained by needle and syringe or indwelling cannula, collected into serum or plasma collection tubes and then processed, centrifuged, stored when appropriate at -80°C, and analyzed.

Concerning reproductive hormones and aldosterone, these blood samples allowed two baseline measurements (with respect to menstrual phase) prior to the start of the training protocol. Regarding the immune factor measures, two baseline measurements of each blood variable also were made prior to the start of the training protocol.

EHT BLOOD COLLECTIONS: HORMONE AND IMMUNE SYSTEM ANALYSES

The EHT tests were conducted before and after the 8-week training program, and on the day following the resting blood collections described above. Pre-exercise and immediate post-exercise blood samples were obtained via indwelling cannula and analyzed for whole blood, plasma, or serum concentrations of cortisol, epinephrine, norepinephrine, lactate, glucose, osmolality, hematocrit, hemoglobin, anti-LPS, and IgG. Analyses of anti-LPS and Total IgG also were performed on 24-hour and 48-hour post-exercise blood samples

REPRODUCTIVE HORMONE AND IMMUNE SYSTEM MEASUREMENTS

Serum estradiol, progesterone, prolactin, TSH, FSH, LH, thyroxin and SHBG were analyzed in the Department of Fertility and Reproductive Endocrinology at New Britain General Hospital via immunoassay (Immulite). This procedure provides excellent sensitivity and reliability by combining highly specific antibodies with enzyme-amplified chemiluminescent chemistry and a proprietary wash technique. Serum aldosterone (19) and cortisol (33) concentrations were analyzed in the Human Performance Laboratory (University of Connecticut) via radioimmunoassay.

CD4 and CD8 determinations were performed on unseparated cells in whole peripheral blood, at the U.S. Army Research Institute of Environmental Medicine (USARIEM), Natick, MA. Briefly, whole heparinized blood was incubated with the specific antibody followed by a fluorescent second antibody. Red blood cells were lysed, the white cells fixed with 1% paraformaldehyde and the samples analyzed by flow cytometry.

IL-10, TNF, and INF-g were measured at USARIEM in plasma by commercially available ELISA kits. CD-14 and CD-45 were also measured, but only to provide data necessary to properly determine CD-4 and CD-8. Anti-LPS IgG was measured by an ELISA produced in-house, and Total IgG by an automated immunoenzyme system (Monarch) at USARIEM (26). We will attempt to measure HSP_{70} via either Western blot or PCR technology at USARIEM in Years II and III. Plasma norepinephrine and epinephrine levels (Year I) were analyzed via an HPLC technique (33).

Hematocrit was determined by microcapillary technique. Hemoglobin was measured by the cyanmethemoglobin method (Kit 525, Sigma Chemical, Inc.) Percent change in plasma volume was calculated using hematocrit and hemoglobin values (20). Osmolality was measured by freezing point depression (model 5004 micro-osmette, Precision Systems, Inc.). Plasma lactate and glucose values were determined using a model 2300 glucose and L-lactate analyzer (Yellow Springs Instruments).

Abbreviations Used in Figures 1 and 2

Figure 1

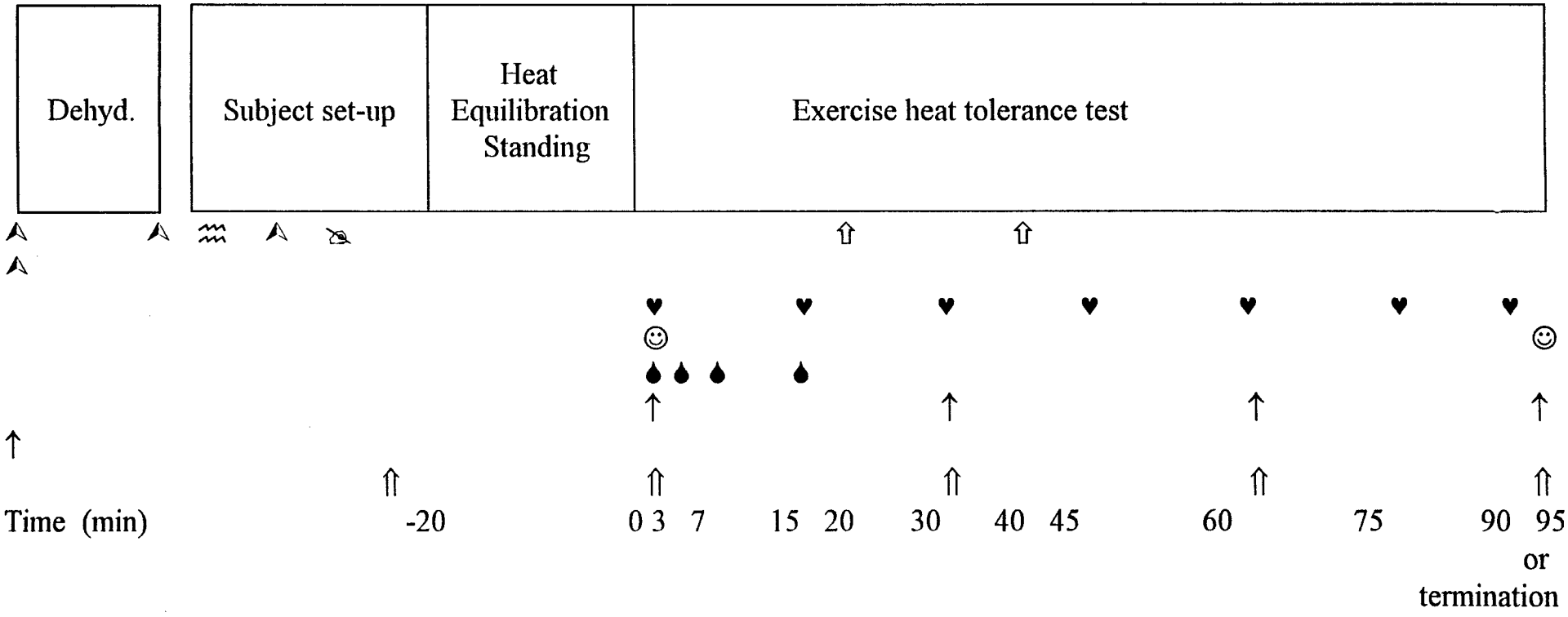
Dehyd. = dehydration to -3 % of body weight
HTT (also EHT) = the exercise-heat tolerance test conducted in our environmental chamber (38°C, 50-70% rh)
V_E = minute ventilation (L/min)
VO_2 = oxygen consumption
RER = respiratory exchange ratio (CO_2/O_2)
BP = blood pressure (systolic/diastolic)
MWST = mean weighted skin temperature, taken at four sites
T_{re} = rectal temperature

Figure 2

ALD = aldosterone
CD_x = cluster of differentiation (x = 4,8,14,45)
DP = Depo-Provera subjects
E_2 = estradiol
EE = ethinyl estradiol
EU-OV = eumenorrheic ovulatory subjects
F = follicular phase
FSH = follicle stimulating hormone
HSP_{70} = heat shock protein
IFN-g = interferon N
IG-1 = immunoglobulin 1
IL-x = interleukin (x = 6,10)
L = luteal phase
LH = luteinizing hormone
MPA = medroxyprogesterone acetate
ORAL = oral contraceptive subjects
P_4 = progesterone
PRL = prolactin
SHBG = sex hormone binding globulin
T_4 = thyroxin
TSH = thyroid stimulating hormone

FIGURE 1

Heat Tolerance Test Schematic



Measurements

- ≈ - void bladder and bowel; weigh exercise clothes prior to dressing and following final body weight
- ⊘ - rectal probe and cannula (insert prior to pre HTT & remove prior to post body weight)
- ☺ - blood draw @ following 20 min standing heat equilibration and at termination
- ⇧ - VO_2, V_E, RER @ min 20 & 40
- ♦ - Local chest sweat rate (Dew point sensor) @ min 0, 3, 7 & 15
- ♥ - HR, T_{re}, RPE, Thermal stress, @ min 0, every 15 min & at 89 min or at termination
- ⅄ - Body weight @ pre & post dehydration, pre & post HTT
- ↑ - BP @ min 0, 30, 60 & 90 or temination & 5 min post
- ⇑ - Skin blood flow & MWST (OtoTemp) @ min -25, 0, 30, 60, & 90 or termination

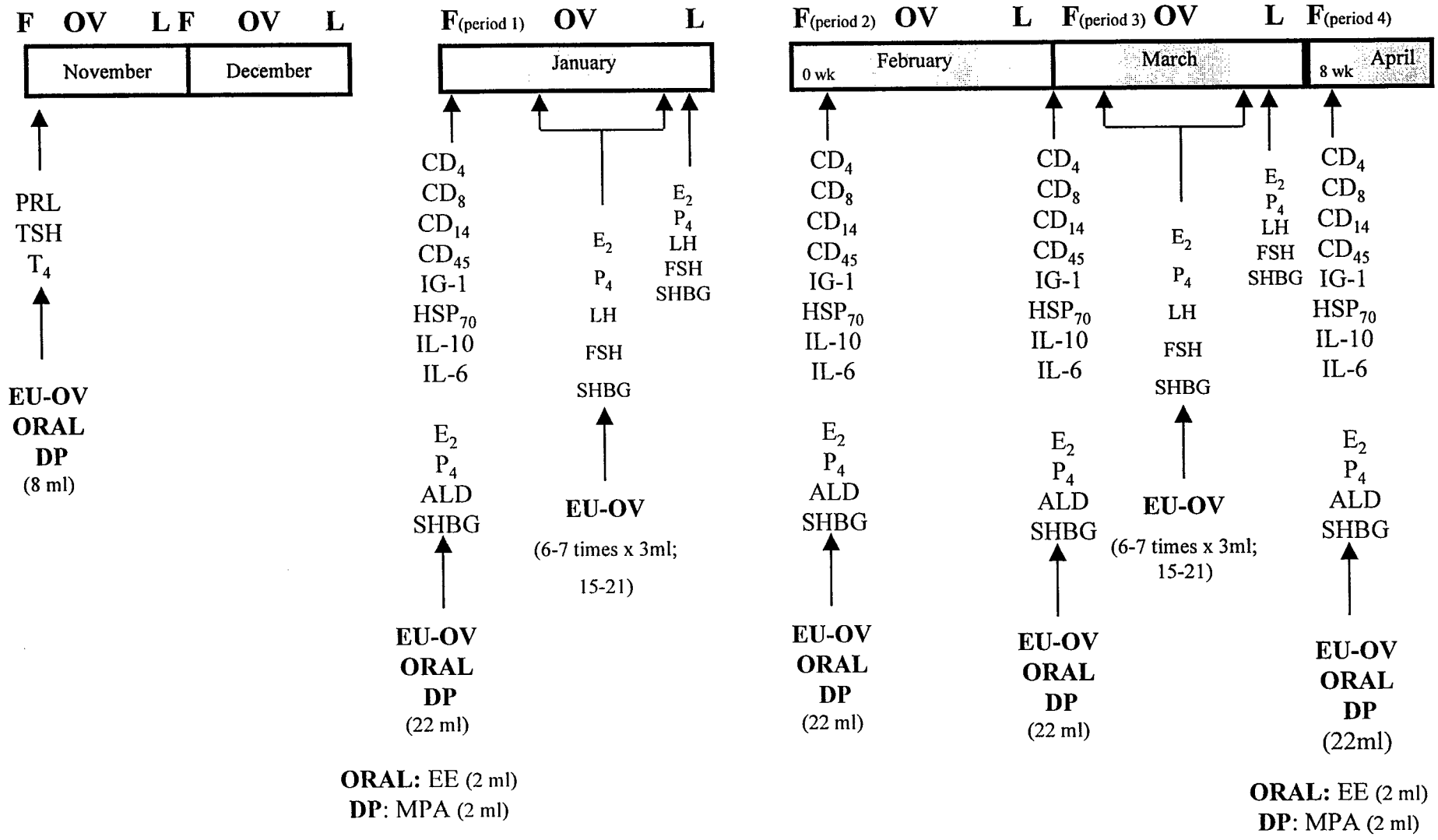


FIGURE 2: Resting blood collection for hormone and immune system measures. (Volumes of blood samples appear at each time point).

STATISTICAL METHODS

In Year II and Year III, data was entered into an IBM computer on the CSS:Statistica™[3.1], Statsoft Data Management System. We will use common descriptive statistics to describe the data sets. In addition, a wide range of multivariate statistics will be used to determine group differences, main effects, interactions and relationships between variables. Appropriate post hoc tests will be performed where significant F ratios are found. Where appropriate (e.g. hormonal response curves), data will also be analyzed by assessing the area under the curve (AUC) response as calculated by the trapezoidal method, after the baseline has been subtracted. Analysis of variance will then be performed on the square root of the AUC. When appropriate, non-parametric analyses also will be used. An alpha level of 0.05 will be used to detect significant differences.

Results and Discussion

YEAR II DATA PRESENTATION

Because the number of subjects in each experimental group was small (n = 1 to 7) during the initial year of testing, most data were presented as group means in the Year I annual report, and no between-group statistical comparisons (i.e., analyses of variance) were attempted. During Year I testing, 12 subjects were tested. During Year II, an additional 11 subjects completed all phases of testing, but one of these was noncompliant and her data were eliminated from the database. This brought the two-year total number of subjects to 22 (i.e., EU-OV, 9; ORAL, 10; DP, 3). Analyses of variance were performed for most between-group comparisons (EU-OV versus ORAL, not including DP) for this Year II annual report, and again will be computed in the Final Report of Year III for all three experimental groups (e.g., EU-OV versus ORAL versus DP).

The DEPO group was not statistically compared to the EU-OV and ORAL groups in any part of this Year II report, due to the small number of subjects tested (n = 3). The mean ± SE values for the DEPO group are presented, without statistical analysis, to allow preliminary comparisons to be made. Year III testing will involve up to 12 additional DEPO subjects; these added subjects will likely alter these means markedly.

Because only three of the 22 test subjects were using Depo Provera (DP), additional efforts are being made during Fall, 1998 to recruit DP users on the University of Connecticut campus and in the surrounding community, for testing in Year III. This will be the primary focus of our recruitment efforts.

The personal characteristics of the 22 test subjects were as follows (mean ± SE): age, 22 ± 1 yr; height, 64 ± 1 cm; body mass, 66.88 ± 2.27 kg; maximal aerobic power, 36.8 ± 0.8 ml O_2/kg/min.

No serious illnesses, serious injuries, or untoward events occurred during Year I or Year II of testing.

REPRODUCTIVE HORMONES: SCREENING & EFFECTS OF 8 WEEKS OF TRAINING

The 22 women who participated in the study represented eumenorrheic women (n=9), oral contraceptive users (n=10) and Depo-Provera users (n=3). All subjects who participated in this investigation passed a hormonal assessment during the screening process. The initial assessment consisted of normal baseline TSH, free T_4, and prolactin. Data for the eumenorrheic group are presented in terms of menstrual cycle milestones, while data for the oral contraceptive group are presented in terms of hormonal assessment, measured during the placebo week of each subject's pill pack. The data of the Depo Provera group, though small in number, are presented separately from the oral contraceptive group in this Year II Annual Report.

Subjects in the eumenorrheic group were assessed for one complete menstrual cycle; this assessment consisted of E_2 , LH, FSH, SHBG, prolactin and P_4 measurements. During the menstrual cycle assessments, blood was drawn during the early follicular phase (days 2-6), the mid-to-late follicular phase (days 8-19), and the mid-luteal phase (5-7 days following the LH peak). Cycle length, ovulation day, normal luteal phase, normal hormonal characteristics and previous cycle lengths were utilized as criteria for admission to the study. Once admitted to the study, eumenorrheic subjects underwent a second complete menstrual cycle assessment during the second month of the 8-week training program.

The oral contraceptive group was evaluated for these same hormones, during the training sessions and following the 8 week training program. Oral contraceptive users had hormonal assessments (estradiol, progesterone, prolactin, SHBG) done during the placebo phase of their pill regimen.

Table 1 - Reproductive Hormones and Menstrual Phase Lengths: Pre- and Post-Training

		Pre-Training	Post-Training
Eumenorrheic Group N=10			
Early Follicular			
	Estradiol	32.1±3.2	27.2±3.8
	Progesterone	0.8±0.1	1.0±0.1
	Prolactin	15.3±2.7	15.9±0.2
	SHBG	43.6±8.4	44.7±5.6
Mid-Cycle			
	Peak estradiol	234.0±24.2	233.1±31.1
	Peak LH	40.8±4.9	39.0±5.1
Mid-luteal			
	Peak estradiol	130.7±18.7	126.9±14.6
	Peak progesterone	10.8±0.7	11.3±0.4
Cycle Parameters			
	Cycle length	27.4±0.8	27.6±0.8
	Ovulation day	15.2±0.6	15.0±0.8
	Follicular length	15.2±0.6	15.0±0.8
	Luteal length	12.1±0.4	12.6±0.5

		Pre-Training	Post-Training
ORAL Contraceptive Group N=10			
	Estradiol	37.6±6.8	30.0±4.5
	Progesterone	0.6±0.1	0.8±0.1
	Prolactin	13.3±2.2	15.9±2.9
	SHBG	159.9±20.0[a]	200.7±27.5[a,b]

		Pre-Training	Post-Training
DEPO Group N=3			
	Estradiol	24.0±4.0	22.7±2.7
	Progesterone	0.3±0.1	0.6±0.2
	Prolactin	16.5±9.2	15.5±6.0
	SHBG	51.5±16.1	50.4±14.8

Abbreviations: LH - luteinizing hormone; SHBG - sex hormone binding globulin.
All values are mean ± SE.
Symbols: a - P<.05, EU-OV versus ORAL; b - pre-training versus post-training (ORAL only)

Table 1 shows that, overall, the three experimental groups responded to the training regimen unremarkably during Year I and Year II testing. The ovulatory status of these groups at post-training appears similar to the pre-training period. Cycle length, follicular phase length, and luteal length appear to have remained unchanged in all groups. The hormonal responses of these subjects also are unremarkable. The greatest between-group difference appears in SHBG, where ORAL levels were higher at both pre- and post-training, as expected. This is likely due to the ingestion of exogenous steroid hormone.

PHYSICAL TRAINING AND HEAT ACCLIMATION (8 WEEKS)

Three days of each week were spent performing stretching, calisthenics (i.e., pushups and situps), and walking/running a 2.85-mi course around campus. The remaining three training days were spent in the Human Performance Laboratory's environmental chamber, performing various types of exercise (i.e., cycling, treadmill walking, bench stepping) in conditions of 37°C, 30-50 %rh; these sessions were designed to induce heat acclimation in all subjects. During exercise-heat acclimation sessions, subjects exercised 72-88 min out of the total 90 min heat exposure.). Subjects did not train one day per week. The proposed 8-week training schedule was accomplished with a very high compliance rate for daily exercise sessions and heat exposures (98.1%, including illness).

The following measurements were made to track the progress of the physical training of test subjects, including:

-- number of pushups and situps (i.e., abdominal crunches) completed in 1 min
-- time to complete the 2.8 mi outdoor course
-- body composition changes (i.e., body mass, % body fat, fat-free mass)
-- maximal aerobic power (VO_{2max}).

The physical training criterion variables appear in **Table 2** below, as recorded during the initial and final weeks of this training program. Values reflect group means ± SE. Column four indicates which values were significantly different (pre- versus post-training/acclimation), as assessed by paired t-tests. These measurements indicate that the 22 test subjects were stronger, more physically fit, and leaner at the end of the 8-week physical training program. There were no significant differences between ORAL and EU-OV, for any variable in **Table 2**.

EXERCISE-HEAT TOLERANCE (EHT) TESTING

Specialized EHT tests were administered to each subject, before and after the 8-week training period. EHT tests consisted of walking on a motorized treadmill at 93.6 $m \cdot min^{-1}$ and 5% grade. The mean temperature and relative humidity were 37°C and 30-50 %rh, with an air flow of 2.3 $m \cdot s^{-1}$. To enhance the stress associated with the EHT, subjects underwent exercise combined with a period of 24-hour water restriction prior to testing, providing approximately -2.9% level of dehydration during the EHT. No water was consumed during the EHT.

The values recorded during the pre-training and post-training EHT tests appear below in **Table 3**, expressed as group means ± SE. Column four indicates which values were significantly different across time (pre- versus post-acclimation), as assessed by paired t-tests.

Each of the measurements in **Table 3** indicate that the 22 test subjects achieved heat acclimation at the end of the 8-week training program. There were no significant differences between ORAL and EU-OV, for any of these variables (pre- versus post-acclimation).

Interestingly, the whole-body sweat rate (i.e., the difference in body mass) tended to be lower during the post-acclimation EHT in ORAL and EU-OV. This likely occurred because the rectal temperature was lower (during post-acclimation testing), thereby stimulating a lower efferent sweating response at the skin.

Skin blood flow was measured with the laser doppler flowmeter, at a site on each subject's forearm. When considered with local sweat rate (below), this data can be used to evaluate heat dissipation in each experimental group. **Table 4** presents the results of skin blood flow measurements. Values represent the percent change of skin blood flow (SBF), from a resting baseline condition (cool 23°C environment) to an exercise-induced state (hot 37°C environment). For example, SBF during the pre-acclimation EHT for the ORAL group was 60.5% greater during exercise than at baseline. Baseline SBF was performed dehydrated, prior to the EHT, in a 23 °C ambient temperature. Exercise SBF was performed at the 15 min point of the EHT, in 37°C ambient temperature. No statistical comparisons were performed for this variable, due to small sample sizes.

Table 2. Physical Training Variables

MEASUREMENT (units)	INITIAL WEEK *	FINAL WEEK	STAT. SIGNIF.
Pushups (per 60 sec)			
ORAL	19 ± 3	39 ± 4	#
EU-OV	11 ± 2	29 ± 4	#
DEPO	18 ± 14	37 ± 12	
Situps (per 60 sec)			
ORAL	46 ± 4	75 ± 5	#
EU-OV	48 ± 6	70 ± 6	#
DEPO	47 ± 4	72 ± 6	
2.8 mile run time (min)			
ORAL	43.7 ± 0.7	32.7 ± 2.2	#
EU-OV	42.7 ± 1.9	29.2 ± 1.1	#
DEPO	43.5 ± 2.3	32.5 ± 2.6	
Body Mass (kg)			
ORAL	66.4 ± 3.4	66.0 ± 3.2	
EU-OV	65.1 ± 3.2	65.1 ± 3.0	
DEPO	73.9 ± 8.6	73.6 ± 8.5	
Body Fat (%) **			
ORAL	27.6 ± 1.7	25.5 ± 1.7	#
EU-OV	28.9 ± 1.3	27.5 ± 1.1	#
DEPO	28.3 ± 6.0	26.4 ± 6.2	
Fat Free Mass (kg) **			
ORAL	47.4 ± 1.4	48.5 ± 1.4	#
EU-OV	46.0 ± 1.7	46.9 ± 1.7	#
DEPO	46.7 ± 2.7	47.8 ± 3.0	
$VO_{2\,max}$ ($l \cdot min^{-1}$)			
ORAL	2.44 ± 0.07	2.71 ± 0.07	#
EU-OV	2.42 ± 0.10	2.65 ± 0.09	#
DEPO	2.52 ± 0.23	2.78 ± 0.15	
$VO_{2\,max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)			
ORAL	37.2 ± 1.3	41.6 ± 1.1	#
EU-OV	37.2 ± 1.1	40.8 ± 0.9	#
DEPO	34.3 ± 2.0	37.9 ± 2.4	

All Data are reported as mean ±SE. Abbreviations: ORAL - Oral Contraceptive Group (n = 10); EU-OV - Eumenorrheic Group (n = 9); DEPO - Depo Provera Contraceptive Group (n = 3); $VO_{2\,max}$ – maximal aerobic power. Symbols: * – pre-training; ** – derived from hydrostatic weighing; # = $P < 0.05$, Pre vs. Post Training

Table 3. EHT Physiological Variables

MEASUREMENT (units)	PRE-ACCLIMATION	POST-ACCLIMATION	STAT. SIGNIF.
Pre-exercise dehydration (% loss)			
ORAL	-2.7 ± 0.2	-2.9 ± 0.2	
EU-OV	-2.9 ± 0.2	-2.9 ± 0.2	
DEPO	-3.0 ± 0.1	-2.8 ± 0.1	
Exercise time to exhaustion (min) *			
ORAL	42.7 ± 5.9	73.4 ± 7.0	#
EU-OV	41.6 ± 3.8	74.9 ± 4.4	#
DEPO	29.0 ± 4.4	63.0 ± 9.5	
Final heart rate (beats/min) **			
ORAL	184 ± 2	156 ± 5	#
EU-OV	184 ± 2	158 ± 3	#
DEPO	184 ± 7	152 ± 7	
Final rectal temperature (°C) **			
ORAL	38.5 ± 0.1	38.2 ± 0.1	#
EU-OV	38.7 ± 0.1	38.3 ± 0.2	#
DEPO	38.2 ± 0.3	38.1 ± 0.1	
Final mean skin temperature (°C) **			
ORAL	35.4 ± 0.3	34.5 ± 0.3	#
EU-OV	35.4 ± 0.1	34.2 ± 0.3	#
DEPO	35.1 ± 0.5	34.5 ± 0.3	
Final rating of perceived exertion **			
ORAL	18 ± 1	13 ± 1	#
EU-OV	17 ± 1	14 ± 1	#
DEPO	18 ± 1	13 ± 1	
Final whole-body sweat rate (L/h)			
ORAL	0.9 ± 0.1	0.8 ± 0.1	
EU-OV	0.9 ± 0.1	0.7 ± 0.1	#
DEPO	0.9 ± 0.1	1.0 ± 0.2	

All Data are reported as mean ±SE. Abbreviations: ORAL - Oral Contraceptive Group (n = 10); EU-OV - Eumenorrheic Group (n = 9); DEPO - Depo Provera Contraceptive Group (n = 3); $VO_{2\,max}$ – maximal aerobic power. Symbols: # = $P < 0.05$, Pre- versus Post-Training; * – or reaching prescribed safety limits of rectal temperature, heart rate, etc.; ** – at same time point in both tests (i.e., the endpoint of the pre-acclimation EHT)

Table 4. Exercise-Induced Skin Blood Flow Change During EHT (% Change from Baseline)

MEASUREMENT	PRE-ACCLIMATION	POST-ACCLIMATION
ORAL	60.5 ± 46.0	54.3 ± 33.1
EU-OV	107.6 ± 37.1	94.9 ± 33.8
DEPO	99.7 ± 28.2	35.7 ± 39.3

All Data are presented as means ±SE. No statistical comparisons were performed for this variable, due to small sample sizes. Abbreviations: ORAL - Oral Contraceptive (n = 3); EU-OV - Eumenorrheic-Ovulatory (n = 6); DEPO - Depo Provera (n = 2).

LOCAL SWEATING RESPONSES

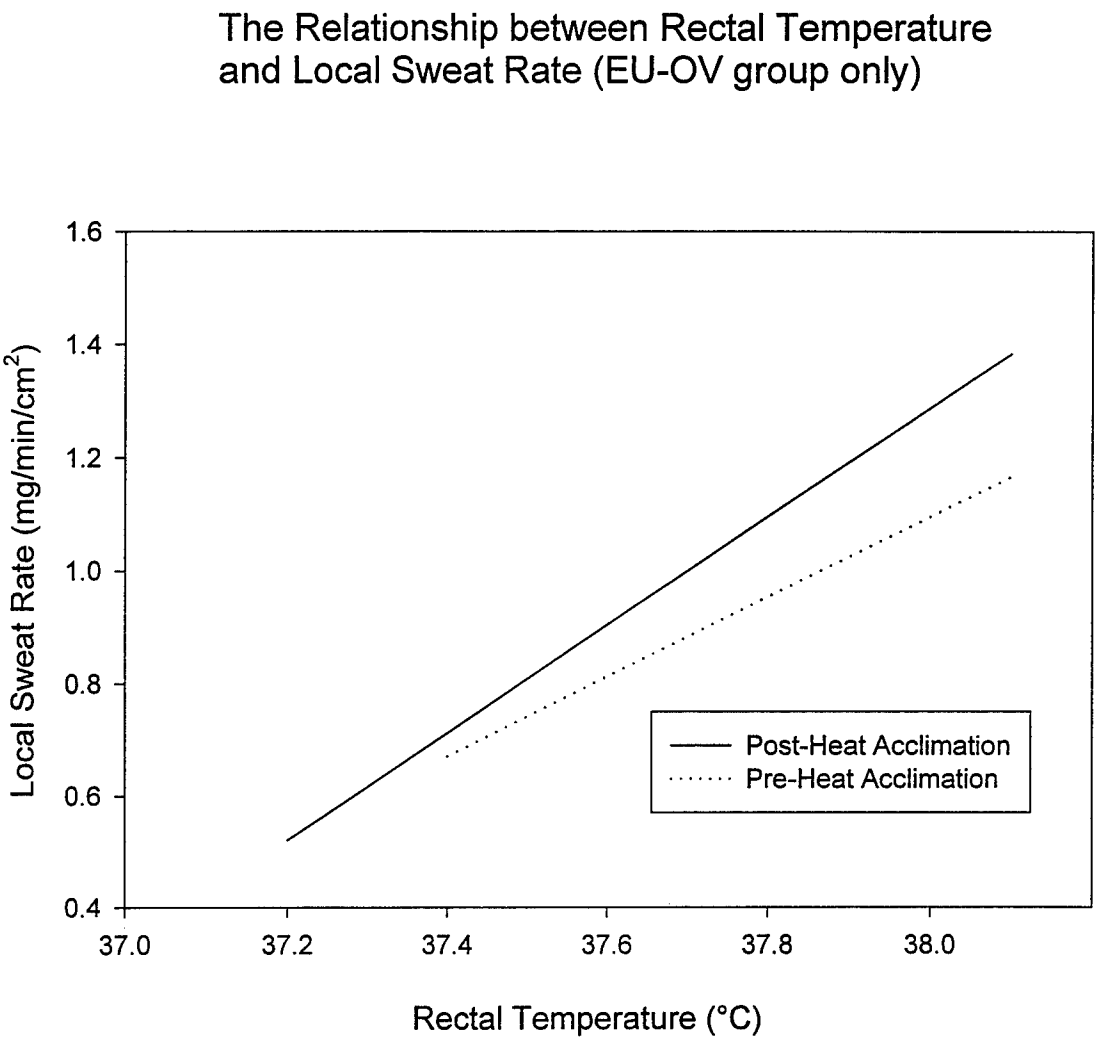
Local sweat rate was measured with a dew point sensor, placed on each subject's back, before and after the 8-week training/acclimation period. Measurements were made in a 23 °C ambient temperature, while subjects exercised on a cycle ergometer. Their body water was normal (euhydrated). Two variables were measured: the threshold temperature for the onset of sweating, and the slope of the line representing the relationship between rectal temperature and local sweat rate (e.g., sweat sensitivity). **Table 5** presents these two variables, as measured before and after the 8-week training/acclimation period. **Figure 3** depicts the relationship between rectal temperature and local sweat rate (EU-OV group only), as measured before and after the 8-week acclimation/training period. Thus, the sweat sensitivity of EU-OV (e.g., the slope of this relationship) apparently increased (e.g., became steeper) as a result of heat acclimation and physical training.

Table 5. Local Sweating: Pre- vs. Post-Acclimation

MEASUREMENT	PRE-ACCLIMATION	POST-ACCLIMATION
Threshold Temperature* for the Onset of Sweating (°C)		
ORAL	37.8 ± 0.1	37.5 ± 0.1
EU-OV	37.6 ± 0.1	37.4 ± 0.1
DEPO	37.7 ± 0.1	37.6 ± 0.1
Sweat Sensitivity (slope)		
ORAL	3.10 ± 0.50	2.13 ± 0.74
EU-OV	1.47 ± 0.66	2.28 ± 0.31
DEPO	2.09 ± 0.11	1.83 ± 0.67

Abbreviations: * - rectal temperature; ORAL - Oral Contraceptive (n = 2); EU-OV - Eumenorrheic-Ovulatory (n = 5); DEPO - Depo Provera (n = 2). All Data are presented as mean ±SE. No statistical comparisons were performed for this variable, due to small sample sizes.

Figure 3


The Relationship between Rectal Temperature and Local Sweat Rate (EU-OV group only)
Local Sweat Rate (mg/min/cm2)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
37.0
37.2
37.4
37.6
37.8
38.0
Rectal Temperature (°C)
Post-Heat Acclimation
Pre-Heat Acclimation

EXERCISE-HEAT TOLERANCE (EHT) TESTING (cont.)

Table 6 contains values for the following variables, measured in plasma during the EHT tests (pre- and post-training): The % change in plasma volume (%CHGPV), glucose, lactate, osmolality, cortisol, epinephrine, and norepinephrine. Values from pre-EHT and post-EHT blood samples are presented. Plasma epinephrine and norepinephrine measurements were performed at the Core Endocrine Laboratory of the Hershey Medical Center, Hershey, PA, under the direction of Dr. Lawrence Demers. All other values were determined at the Human Performance Laboratory, University of Connecticut.

The results in **Table 6** demonstrate that 8 weeks of exercise training and heat acclimation had significant effects ($P<0.05$) on some plasma variables, which may lead to specific conclusions after testing in Year III. These differences are described below.

Plasma Glucose: Only the ORAL group showed a significant increase in glucose concentration, during the pre-acclimation EHT test. It is possible that this is a Type I statistical error; this will be evaluated after additional ORAL subjects have been tested in Year III testing.

Plasma Lactate: Reductions in the post-exercise lactate levels appeared after 8 weeks, in both the ORAL and EU-OV groups. The DEPO group showed a tendency to respond in the same manner. These responses were likely due to physical training and/or heat acclimation.

Plasma Osmolality: As expected, the dehydration and fluid shifts which occurred during the EHT test caused osmolality to rise, in both pre- and post-acclimation trials, for ORAL and EU-OV.

Plasma Cortisol: This variable shows the most obvious between-group differences of any plasma constituent. ORAL consistently exhibited higher cortisol levels than EU-OV. At the time of writing this report, the analytical cross-reactivity of oral contraceptives (e.g., exogenous steroids) with the cortisol assay is unknown, but we will investigate this possibility.

Plasma Norepinephrine: As anticipated, plasma norepinephrine was a more sensitive marker of heat stress (i.e., pre- versus post-exercise increases) than plasma epinephrine. This has been shown in several previous studies, in the Human Performance Laboratory at the University of Connecticut and in the (former) Heat Research Division of USARIEM. Several *between-group* (ORAL versus EU-OV) and *across-time* differences in norepinephrine levels were identified. These will be evaluated fully in the Year III Final Report, but currently suggest that the ORAL group exhibited a lower pre-exercise value during the post-acclimation EHT test. The mechanism for this decrease in circulating norepinephrine level is presently not known.

RESTING ALDOSTERONE LEVELS IN BLOOD

Aldosterone levels were measured at rest, in early morning blood samples, at three time points: before (PRE), at 4 weeks (MID), and at 8 weeks of physical training (POST). **Table 7** demonstrates that there were no statistically significant differences in circulating ALDOSTERONE levels, either between the ORAL and EU-OV groups or across time in either group.

IMMUNOLOGICAL FACTORS

The analyses performed during Year I and Year II were as follows:

1. Lymphocyte phenotypes of CD4+ (measure of T_{helper} cells) and CD8+ (measure of $T_{suppressor/cytotoxic}$ cells) by Flow Cytometry
2. Anti-Lipopolysaccharide Immunoglobulins (Anti-LPS) by ELISA
3. Total IgG by Monarch immunoassay.

In addition, the following analyses were performed during Year I, but were not analyzed by the time this Year II report was prepared:

4. Circulating cytokines by ELISA kits.

All of the above analyses were performed on EDTA-preserved blood samples that were collected, kept in an insulated container at room temperature and either transported immediately to USARIEM for processing for flow cytometric studies, or processed into plasma and stored at -80°C until all samples were collected and then brought frozen to USARIEM for determination of Anti-LPS, Total IgG and Cytokines.

Table 6. EHT Blood Variables

MEASUREMENT (units)	PRE-ACCLIMATION		POST-ACCLIMATION		STAT.
	PRE-EHT	POST-EHT	PRE-EHT	POST-EHT	SIGNIF.
%CHGPV (%)					
ORAL		-2.0 ± 1.2		-3.0 ± 1.3	
EU-OV		-5.7 ± 1.1		-4.0 ± 1.6	
DEPO		-5.4 ± 0.4		-4.6 ± 1.3	
plasma glucose (mg/dl)					
ORAL	96.1 ± 3.7	112.1 ± 5.1	97.7 ± 4.2	110.2 ± 7.7	#
EU-OV	105.7 ± 5.9	101.4 ± 3.2	102.2 ± 4.1	109.1 ± 4.0	
DEPO	90.6 ± 2.7	120.6 ± 8.3	92.1 ± 2.0	118.9 ± 18.4	
CV = 0.9%					
plasma lactate (mmol)					
ORAL	1.1 ± 0.1	2.0 ± 0.2	1.3 ± 0.2	1.4 ± 0.1	# *
EU-OV	1.3 ± 0.2	2.4 ± 0.5	1.2 ± 0.2	1.9 ± 0.3	# ‡
DEPO	1.0 ± 0.1	2.3 ± 0.1	0.9 ± 0.1	1.5 ± 0.4	
CV = 1.7%					
plasma osmolality (mosmol/l)					
ORAL	290.7 ± 0.8	296.6 ± 1.3	288.8 ± 1.3	297.9 ± 1.4	# ‡
EU-OV	292.7 ± 1.1	296.3 ± 1.5	289.7 ± 1.1	297.8 ± 1.1	# ‡
DEPO	286.5 ± 0.9	293.0 ± 1.0	287.5 ± 1.3	296.7 ± 2.2	
CV = 0.2%					
plasma cortisol (nmol/l)					
ORAL	845 ± 51	828 ± 88	822 ± 38	857 ± 97	
EU-OV	487 ± 84	517 ± 59	354 ± 40	675 ± 83	‡
DEPO	442 ± 121	715 ± 137	326 ± 78	539 ± 39	
CV = 7.7%	£	£	£		
plasma epinephrine (pg/ml)					
ORAL	117 ± 40	47 ± 16	108 ± 51	125 ± 62	
EU-OV	117 ± 65	164 ± 55	34 ± 4	176 ± 82	
DEPO	41 ± 20	45 ± 20	34 ± 7	29 ± 7	
CV = 15.0%					
plasma norepinephrine (pg/ml)					
ORAL	344 ± 40	976 ± 194	200 ± 12	834 ± 113	# ‡ †
EU-OV	314 ± 23	1233 ± 153	297 ± 30	1154 ± 153	# ‡
DEPO	279 ± 19	1282 ± 232	239 ± 59	1097 ± 427	
CV = 9.0%			£		

£ - P<0.05 Between ORAL and EU-OV groups only, at that time point.
- P<0.05 within Pre-Acclimation (PRE-EHT vs. POST-EHT)
‡ - P<0.05 within Post-Acclimation (PRE-EHT vs. POST-EHT)
† - P<0.05 Pre-Acclimation (PRE-EHT) vs. Post-Acclimation (PRE-EHT)
* - P<0.05 Post-Acclimation (POST-EHT) vs. Post Acclimation (POST-EHT)
CV - interassay coefficient of variation for that analysis; all runs for a given subject were completed within the same assay, to minimize the interassay varability.

Table 7. Morning Blood Levels of Aldosterone (at rest)

MEASUREMENT (units)	PRE	MID	POST
Aldosterone (pmol/l)			
ORAL	910 ± 108	640 ± 89	748 ± 96
EU-OV	671 ± 168	599 ± 137	582 ± 93
DEPO	586 ± 225	588 ± 70	707 ± 66
CV = 10.34%			

Abbreviations: PRE - before physical training began; MID - at 4 weeks of physical training; POST - after 8 weeks of physical training had been completed; ORAL - Oral Contraceptive (n = 10); EU-OV - Eumenorrheic-Ovulatory (n = 9); DEPO - Depo Provera (n = 3); CV - interassay coefficient of variation for that analysis; all runs for a given subject were completed within the same assay, to minimize the interassay varability. No significant differences existed across time or between groups (ORAL versus EU-OV).

IMMUNOLOGICAL FACTORS (cont.)

It was not possible to perform IFNg AND IL-10 analyses before the deadline of this report, because of technical/training/manpower difficulties. These determinations will be added during Year III, as described below in the section titled "Recommendations Regarding the Statement of Work".

None of the Flow cytometry, Anti-LPS IgG, or Total IgG data (below in **Figures 4 - 9**) were subjected to statistical analysis at the time of publication of this Year II report. The DEPO group is not included in the comments below, due to the small number of subjects in that group (n = 3).

Flow Cytometry: See **Figure 4**. Little change occurred in *CD4+ levels* across time. Because of the great overlap in standard error terms, it is unlikely that CD4+ differences exist between groups. *CD8+ levels* apparently did not change across time but, in contrast to CD4+ levels, suggested that the ORAL group suppressed CD8+ lymphocyte production, at all time periods (not statistically verified). It is possible that the use of oral contraceptives depressed CD8+ production. The *CD4/CD8 ratio* exhibited a trend in which the ORAL group had a higher CD4/CD8 ratio (versus EU-OV, except at period 3), due to lower CD8+ levels in ORAL. Further, it is possible in EU-OV subjects that 4 weeks of training and heat acclimation increased immune status slightly (see Period 3). These trends in the CD4/CD8 ratio will be fully evaluated in the Final Report for this project.

Anti-LPS IgG: See **Figure 5**. These preliminary data show a trend (not statistically verified) for subjects in the ORAL and DEPO groups to have lower circulating levels of Anti-LPS than EU-OV subjects. It is possible that these exogenous steroid hormones offered a protective effect on the gut lining, thereby reducing the entry of LPS into the blood and reducing the amount of LPS antibody produced. Corticosteroids have been shown to have a prophylactic effect on gut lining integrity, and to reduce LPS entry into the circulation of hyperthermic primates (28). Also, it is not known whether the progressive rise in Anti-LPS, over the course of testing, is statistically significant. Increased sample sizes and statistical analyses will test this hypothesis in our Year III Final Report.

Total IgG: See **Figure 6**. Total IgG is very similar in the ORAL and EU-OV subjects, during the initial four periods of blood sampling. After training began (periods 3, 4, 4a, 4b), the subjects using oral contraceptives exhibited a trend toward higher Total IgG antibody levels (likely not statistically significant, at present). This suggests that oral contraception improved immune status. Increased sample sizes and statistical analyses will test this hypothesis in the Final Report of Year III.

IL-10: **Figure 7** presents preliminary data (i.e., not all analyses have been completed) from 3 subjects in ORAL (numbers 35, 40, 44) and 2 subjects in EU-OV (numbers 53, 55). Due to the small sample sizes and absence of any DEPO subjects, trends (and treatment effects) are impossible to discern.

INF-g: See **Figure 8** (all experimental groups combined). This figure appeared in the Year I report; it is presented here because it was not possible to perform the IFNg analyses for Year II before the deadline of this report, due to of technical/training/manpower difficulties. The Year II and Year III determinations will be presented in the Year III Final Report. **Figure 8** shows that, when the concentration of INF-g was plotted against the time point in the study protocol, a correlation of r = 0.99 was observed. This suggests a progressive increase in INF-g during the 8-

week experimental protocol, from baseline to post-training. Although not tested statistically, this trend is very interesting and deserves further analysis, in Year II and Year III. INF-g and IL-10 are considered among the best factors to monitor the state of cytokine production because INF-g blocks the production of the Th2 cytokines (i.e., immunostimulatory factors such as IL-4, IL-5, IL-6, IL-9, IL-10, IL-13), while IL-10 down-regulates the production of Th1 (i.e., immunosuppressive) cytokines.

TNFa: The cytokine Tumor Necrosis Factor-alpha (TNF_a) was measured in Year I (see **Figure 9,** all experimental groups combined). No changes in TNF-a were observed in Year I. Because cytokine assays are very expensive, analytical funds were severely limited, and because the relevance of TNFa as an index of immune status has been questioned, we decided to eliminate the TNFa determinations and concentrate on the more important INFg and IL-10 analyses (see below).

Recommendations Regarding the Statement of Work

1. Because only three of the 12 test subjects were using Depo Provera, special efforts are currently underway to recruit Depo Provera users on the University of Connecticut campus and in the surrounding community for testing in Year III. These efforts include the posting of fliers specific to Depo-Provera users, identification of Depo-Provera users by the Student Health Services staff, recruiting at off-campus counseling centers, and specific recruiting by physicians in the surrounding community. We foresee no difficulty in securing additional eumenorrheic women and oral contraceptive users.

2. The timing of receipt of funding, ordering through the university procurement system, and delivery of two instruments (i.e., laser doppler and dew point sensor) made it impossible to use these instruments in Year I pre-training tests. Measurements of skin blood flow and local sweat rate were made in Year II and appear in this report. These measurements also will be made during Year III.

Due to the capacities and idiosyncrasies of these instruments, pilot studies (Fall, 1997) determined that it was better to measure the PRE-EHT skin blood flow in a cool environment (23°C, outside the environmental chamber) and to measure skin blood flow following 15 min of exercise in the heat (37°C, inside the environmental chamber). This allows the change (delta) in skin blood flow to be calculated from rest to exercise. Other pilot studies (Summer, 1997) determined that the *onset temperature* and the *slope* of local sweating (i.e., the relationship between rectal temperature and sweat rate) were best measured during exercise in a cool/mild environment, because of saturation of the dew-point sensor capsule. These data will provide information regarding the effects of training and heat acclimation on heat dissipation and temperature regulation.

3. During 1997, the determinations of tumor necrosis factor-alpha (TNFa), interferon gamma (INFg), and interleukin-10 (IL-10) indicated that these cytokines displayed no specific trends or significant differences. Because cytokine assays are very expensive, analytical funds were severely limited, and because the relevance of TNFa as an index of immune status has been questioned, we decided to eliminate the TNFa determinations and concentrate on the more important INFg and IL-10 analyses (J.L. Rossio. In: Committee on Military Nutrition Research. *Nutrition and Immune Function: Strategies for Sustainment in the Field.* Proceedings of a Workshop, Fort Detrick, MD, 1996. National Academy Press.)

4. A future suggestion regarding analyses at USARIEM: If unlimited funds were available, we would measure intracellular INFg and IL-10, in addition to circulating cytokines.

5. In Year I it was necessary to transport fresh blood samples on a regular basis to both New Britain General Hospital, New Britain, CT and USARIEM, Natick, MA, because those samples could not be frozen and required timely analysis (i.e., within four hours of blood collection). Our original budget did not specify a line item for this travel expenditure, which involved nine different individuals using their privately-owned automobiles for this purpose. A review of the Project Year I budget revealed that approximately $3,000 was used for this purpose. We reported in the Year I Annual Report that it would be ideal to use a courier service for this purpose, to remove the burden and liability of such travel on research personnel. We utilized a courier service to ship blood samples during Year II, at a one-year cost of $2,887.00.

Abbreviations Used in Figures 4 - 9

ORAL - Oral Contraceptive (n = 10)
EU-OV - Eumenorrheic-Ovulatory (n = 9)
DEPO - Depo Provera (n = 3)

Figure 4

CD-4+ = cluster of differentiation #4; CD-8+ = cluster of differentiation #8
Period 1 = Baseline I (~ 1 month pre-training)
Period 2 = Baseline II (pre-training)
Period 3 = Mid-training (~ 4 weeks)
Period 4 = After 8 weeks of training

Figure 5

LPS = lipopolysaccharide (a toxic cell wall component of gram negative bacteria)
anti-LPS = the antibody formed in response to the circulating antigen LPS
Note: All values in this figure were collected during EHT #1 and #2. The dashed line between Period 2b and 3 represents the boundary between pre-training (Periods 1 - 2b) and post-training (Periods 3 - 4b).
Period 1 = Pre-training (before exercise began in EHT #1)
Period 2 = Pre-training (immediately following exercise in EHT #1)
Period 2a = Pre-training (24 h after the exercise of EHT #1)
Period 2b = Pre-training (48 h after the exercise of EHT #1)
Period 3 = Post-training (before exercise began in EHT #2)
Period 4 = Post-training (immediately following exercise in EHT #2)
Period 4a = Post-training (24 h after the exercise of EHT #2)
Period 4b = Post-training (48 h after the exercise of EHT #2)

Figure 6

IgG = immunoglobulin G. The dashed line between Period 2b and 3 represents the boundary between pre-training (Periods 1 - 2b) and post-training (Periods 3 - 4b).
Period 1 = Pre-training (before exercise began in EHT #1)
Period 2 = Pre-training (immediately following exercise in EHT #1)
Period 3 = Pre-training (24 h after the exercise of EHT #1)
Period 4 = Pre-training (48 h after the exercise of EHT #1)
Period 5 = Post-training (before exercise began in EHT #2)
Period 6 = Post-training (immediately following exercise in EHT #2)
Period 7 = Post-training (24 h after the exercise of EHT #2)
Period 8 = Post-training (48 h after the exercise of EHT #2)

Figure 7

IL-10 = interleukin-10
Period 1 = Baseline I (~ 1 month pre-training)
Period 2 = Baseline II (pre-training)
Period 3 = Mid-training (~ 4 weeks)
Period 4 = After 8 weeks of training

Figure 8

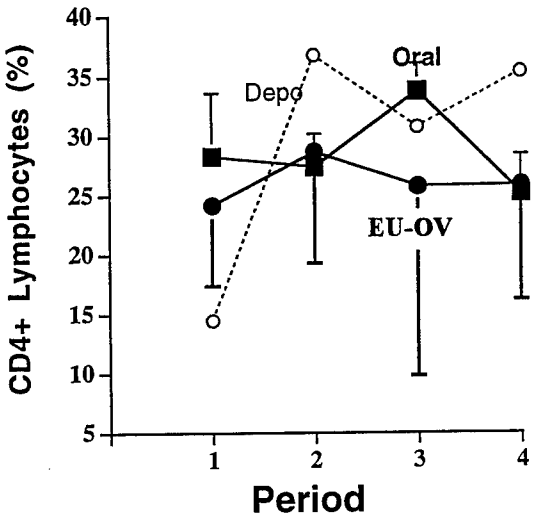
INF-g = interferon gamma (Year I only, all subjects combined, n = 12)
Period 1 = Baseline I (~ 1 month pre-training)
Period 2 = Baseline II (pre-training)
Period 3 = Mid-training (~ 4 weeks)
Period 4 = After 8 weeks of training

Figure 9

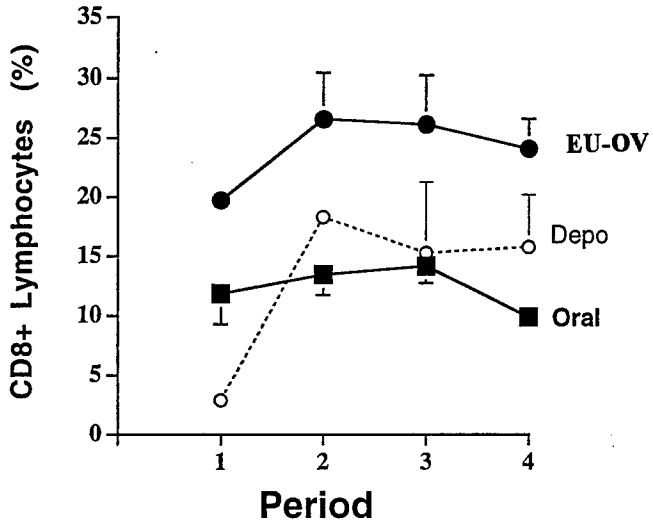
TNFa = tumor necrosis factor-alpha, a cytokine (Year I only, all subjects combined, n = 12)
Period 1 = Baseline I (~ 1 month pre-training)
Period 2 = Baseline II (pre-training)
Period 3 = Mid-training (~ 4 weeks)
Period 4 = After 8 weeks of training

Figure 4

CD4+Lymphocytes



CD8+ Lymphocytes



CD4/CD8 Ratio

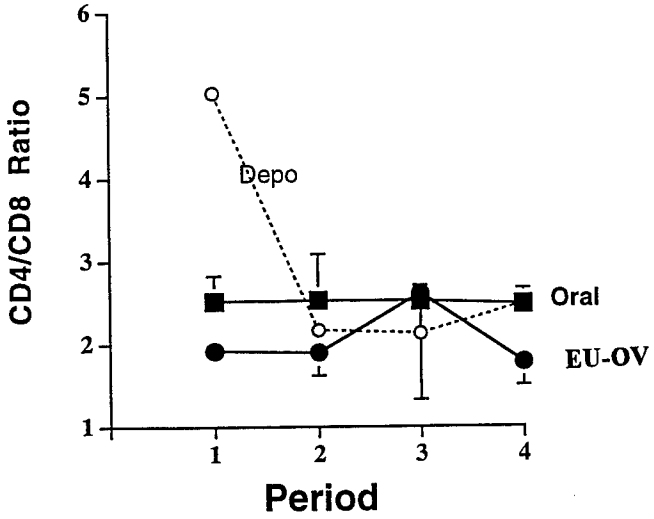


Figure 5

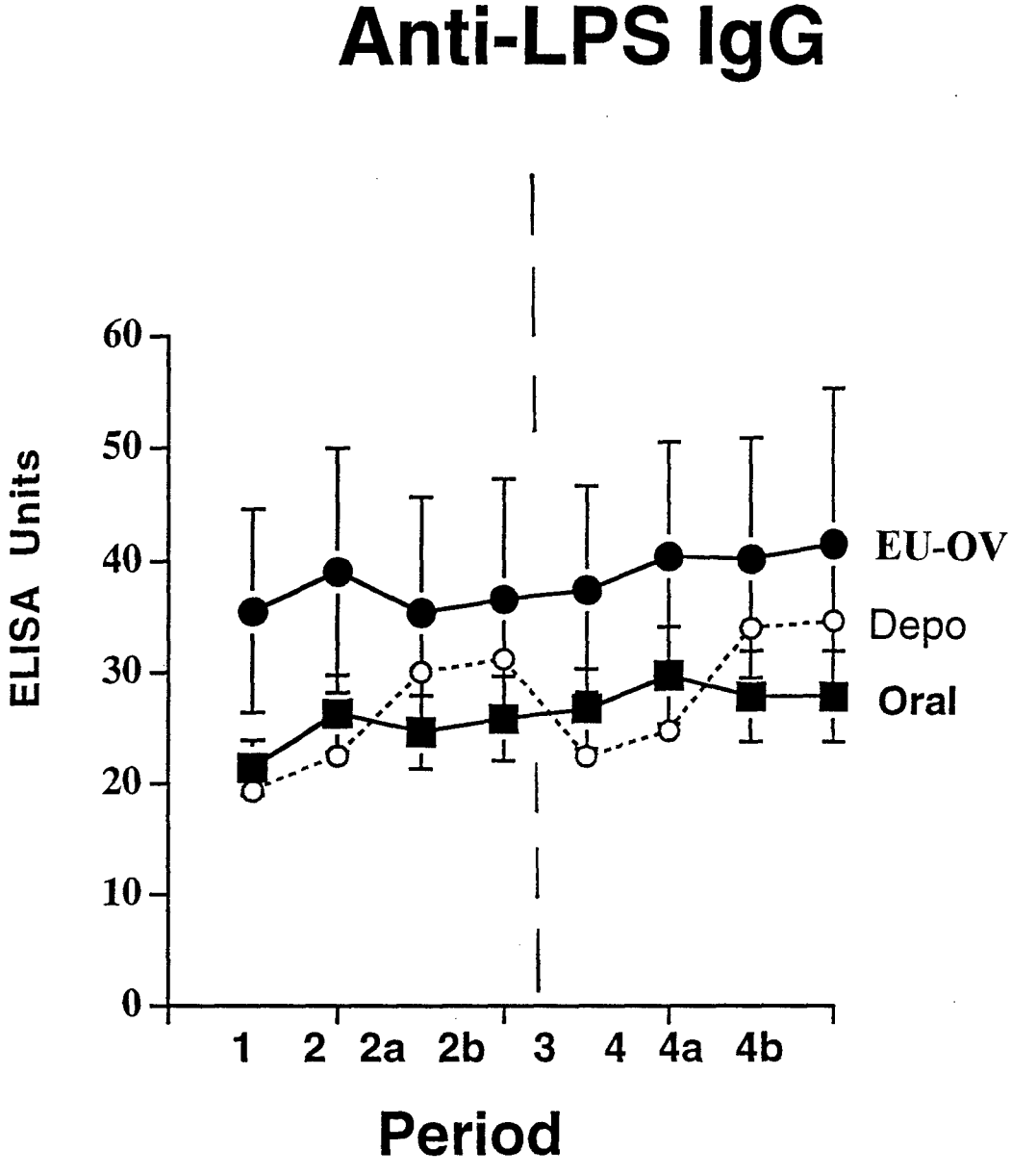

Anti-LPS IgG
ELISA Units
60
50
40
30
20
10
0
1
2
2a
2b
3
4
4a
4b
Period
EU-OV
Depo
Oral

Figure 6

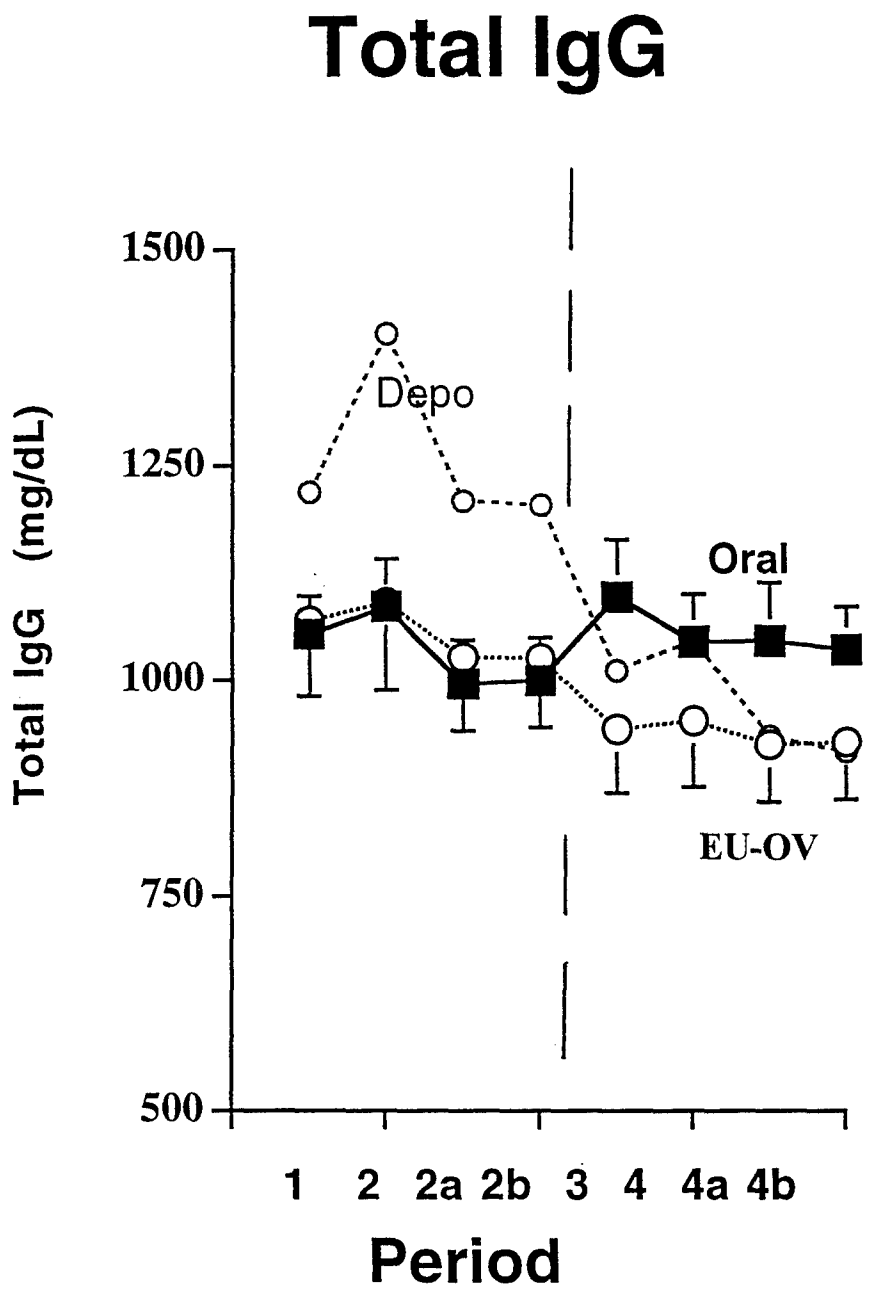

Total IgG
1500
1250
1000
750
500
Total IgG (mg/dL)
Depo
Oral
EU-OV
1
2
2a
2b
3
4
4a
4b
Period

Figure 7

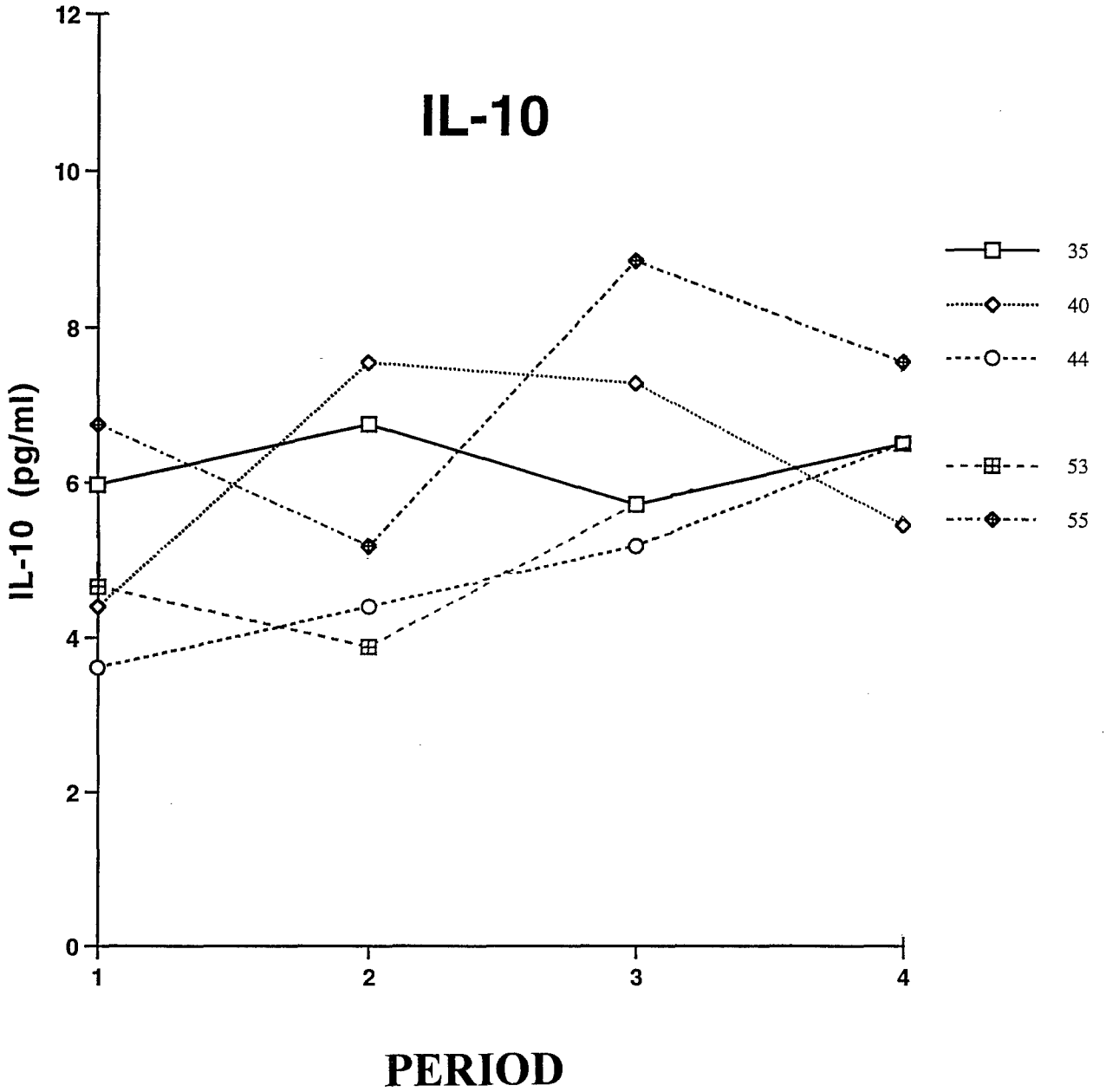


Figure 8

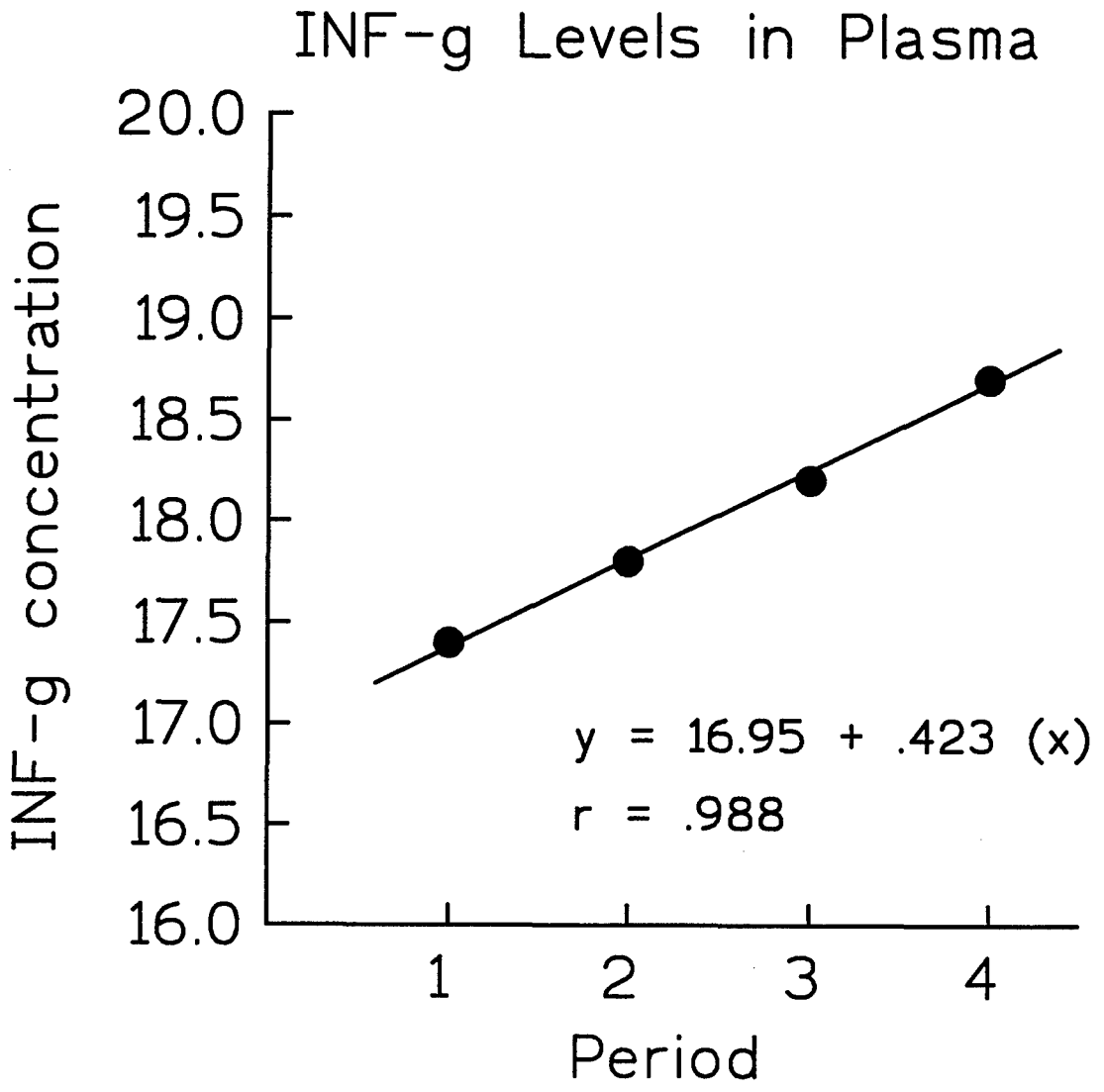
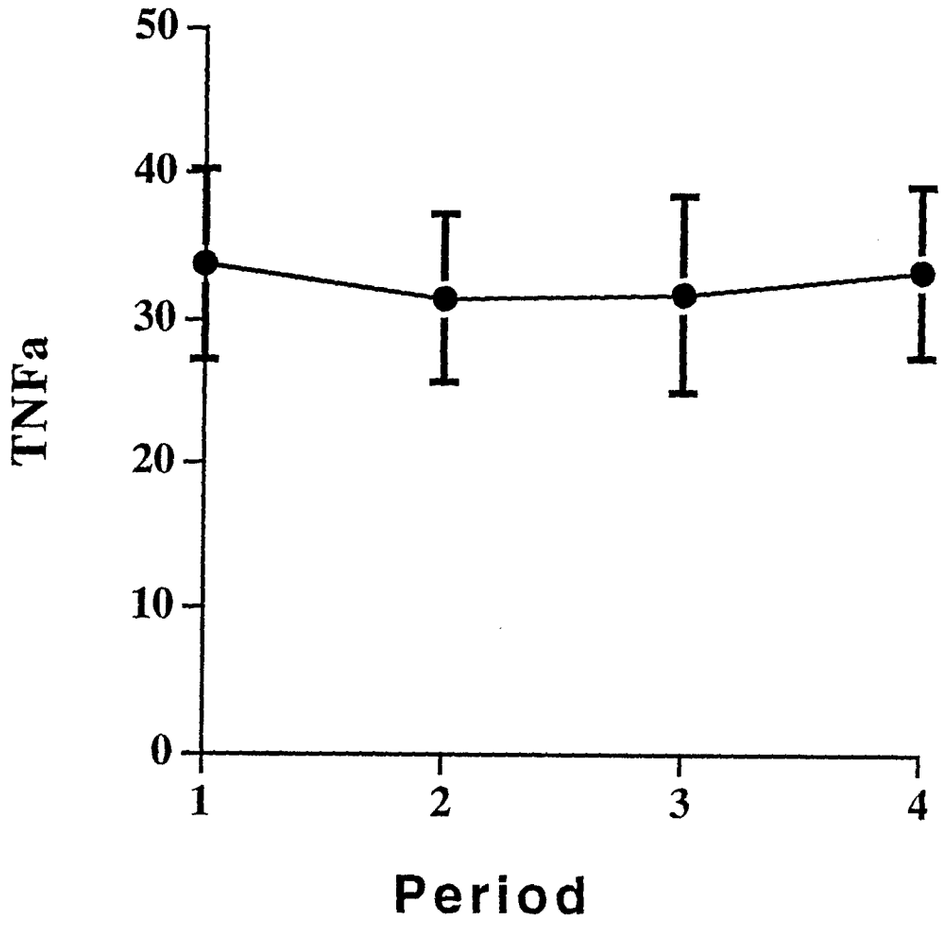

INF-g Levels in Plasma
INF-g concentration
20.0
19.5
19.0
18.5
18.0
17.5
17.0
16.5
16.0
y = 16.95 + .423 (x)
r = .988
1
2
3
4
Period

Figure 9



Conclusions

1. Over 90 female volunteers were originally identified as potential test subjects, but hormonal and other physiological/clinical screening narrowed the pool to 22. These test subjects completed all phases of Year I and Year II testing. Because the number of subjects was small (n = 3), no statistical comparisons have been reported in this Year II Annual Report for the DEPO group. Only means $\pm$ SE are reported, to allow preliminary comparisons.

2. Each of the physical training variables indicate that the 22 test subjects were stronger, more physically fit, and leaner at the end of the 8-week physical training program.

3. Each of the variables measured during EHT tests indicate that the 22 test subjects achieved heat acclimation at the end of the 8-week training program.

4. In terms of reproductive hormone trends, study participants responded to the training regimen unremarkably. The ovulatory status of the eumenorrheic group at post-training appears similar to the pre-training period. Cycle length, follicular phase length, and luteal length appear to have remained unchanged. Hormonal responses of the contraceptive users also appear to be unremarkable.

5. In terms of aldosterone and the stress hormones, results are unremarkable at this time. There were no significant differences across time, or between ORAL and EU-OV, in resting morning aldosterone levels. As anticipated, resting EU-OV cortisol measures were lower than corresponding ORAL values at both Pre-EHT and Post-EHT during the Pre-Acclimation period. This also was evident Post-Acclimation for the Pre-EHT blood measure. However, the EU-OV group demonstrated a reduced plasma cortisol at Pre-EHT, during Post-Acclimation testing (versus Pre-Acclimation testing). Plasma epinephrine values showed no significant differences across time or between the ORAL and EU-OV groups. For plasma norepinephrine, there was a significant increase in circulating levels in response to the EHT, during both the Pre-Acclimation and Post-Acclimation testing. Pre-EHT values were significantly reduced in ORAL, in response to heat acclimation, and these values were lower than the corresponding EU-OV measures.

6. An original health concern of women soldiers during deployment was that the consumption of oral contraceptives might alter their immune systems, placing them at greater risk of infections. To date, trends in the data suggest that oral contraceptives do not depress the immune system but rather moderately activate both the humoral and cellular immune systems. This may mean that oral contraceptives improve the ability of female soldiers to resist infections. Year III observations will clarify this hypothesis.

Individuals Who Received Funds From This Grant During Year II

	Type of Funds *
Responsible Investigators	
Lawrence E. Armstrong, Ph.D.	S, CA, ST
Carl M. Maresh, Ph.D.	S
Master's Students	
Dean Aresco	1/2 GA
Timothy Bilodeau	1/2 GA, ST
Jen Ormerod	ST
Doctoral Students	
Tabatha Elliott	S, ST
Timothy Scheett	1/2 GA, S, ST
James Stoppani	1/2 GA, S, ST
Lori Svetkey	ST

*** - KEY:**

S, summer income

CA, conference attendance: Federation of American Societies for Experimental Biology

ST, sample transport between University of Connecticut and either USARIEM (to Dr. Gaffin) or New Britain General Hospital (to Dr. De Souza)

GA, graduate assistantship (research assistantship) through the University of Connecticut

References

1. Altura BM. Sex and estrogens in protection against circulatory stress reactions. Am J Physiol 1976;231:842-847.
2. Altura BM. Reticuloendothelial cells and host defense. Adv Microcirc 1980;9:252-254.
3. Armstrong LE. Considerations for replacement beverages: fluid-electrolyte balance and heat illness. In: Fluid Replacement and Heat Stress. Marriott B (ed.). Washington, DC: National Academy Press, National Academy of Sciences, Institute of Medicine, Food and Nutrition Board 1994;37-54.
4. Armstrong LE, Costill DL, Fink WJ, Bassett D, Hargreaves M, Nishibata I, King DS. Effects of dietary sodium on muscle potassium content during heat acclimation. Eur J Appl Physiol 1985;54:391-397.
5. Armstrong LE, De Luca JP, Hubbard RW. Time course and heat acclimation aility of prior exertional heatstroke patients. Med Sci Sports Exerc 1990;22:36-48.
6. Armstrong LE, Hubbard RW, Szlyk PC, Sils IV, Kraemer WJ. Heat intolerance, heat exhaustion monitored: a case study. Aviat Space Environ Med 1988;59:262-266.
7. Armstrong LE, Maresh CM. The induction and decay of heat acclimatization in trained athletes. Sports Med (New Zealand) 1991;12:302-312.
8. Baker DA, Salvatore W, Milch PO. Effect of low-dose oral contraceptives on natural killer cell activity. Contraception 1989;39(1):119-124.
9. Bannister RG. Anhidrosis following intravenous bacterial pyrogen. Lancet 1960;ii:118-122.
10. Borg G. Perceived exertion as an indicator of somatic stress. Can J Rehabil Med 1970;2:92-98.
11. Bortoff A. Influence of exercise on gastrointestinal function. In: Eercise, Nurition, and Energy Metabolism. Horton ES, Terjung RL (ed.). New York: Macmillan, 1988;159-171.
12. Bosenberg AT, Brock-Utne JG, Wells MT, Blake GT, Gaffin SL. Strenuous exercise causes systemic endotoxemia. J Appl Physiol 1988;65:106-108.
13. Bouchama A, Parhar RS, Er-Yazigi A, Sheth K, Al-Sedairy S. Endotoxemia and release of tumor necrosis factor and interleukin-1-alpha in acute heatstroke. J Appl Physiol 1991;70:2640-2644.
14. Bouchama A, Al-Sedairy S, Siddiqui S, Shail E, Rezeig M. Elevated pyrogenic cytokines in heatstroke. Chest 1993;104:1498-1502.
15. Brock-Utne JG, Gaffin SL, Wells MT, et al. Endotoxemia in exhausted runners following a long distance race. S A Med J 1988;73:533-536.
16. Cannon JG. Exercise and resistance to infection. J Appl Physiol 1993;74:973-981.
17. Costill DL, Fox E. Energetics of marathon running. Med Sci Sports 1969;1:86-91.
18. Culbertson Al, Rosenfeld P. Assessment of sexual harassment in the active-duty Navy. Mil Psychol 1994;6:69-93.
19. De Souza MJ, Maresh CM, Maguire MS, Kraemer WJ, Flora-Ginter G, Goetz KL. Menstrual status and plasma vasopressin, renin activity and aldosterone exercise responses. J Appl Physiol 1989;67:736-743.
20. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974;37:247-248.
21. Dinarello Ca, Cannon JG, Wolff SM, et al. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin-1. J Exp Med 1986;163:1433-1450.
22. Earl DT, David DJ. Depo-Provera: an injectable contraceptive. Am Family Physician 1994;49:891-894.
23. Elson CO, Beagley KW. Cytokines and immune mediators. In: Physiology of the Gastrointestinal Tract. Johnson LR (ed.). New York: Raven Press, 1994;243-265.
24. Epstein Y. Heat intolerance: predisposing factor or residual injury? Med Sci Sports Exerc 1990;22:29-35.
25. Forrest JD. Epidemiology of unintended pregnancy and contraceptive use. Am J Obstet Gynecol 1994;170:1485-1488.
26. Gaffin SL, Badsha N, Brock-Utne JG, Vorster B, Conradie J. An ELISA procedure for detecting human anti-endotoxin antibodies in serum. Ann Clin Chem 1982;19:191-196.
27. Gaffin SL, Gentile B, Koratich M, Leva N, Hubbard RW, Francesconi RP. Heatstroke in a miniswine model. Shock, in press, 1995.
28. Gaffin SL, Hubbard RW. Experimental approaches to therapy and prophylaxis for heat stress and heatstroke. J Wilderness Med, in press, 1995.
29. Gathiram P, Wells MT, Brock-Utne JG, Gaffin SL. Portal and systemic arterial plasma lipopolysaccharide concentrations in heat stressed primates. Circ Shock 1988;25:223-230.

30. Ghezzi P, Dinarello CA, Bianchi M, Rosandich ME, Repine JE, White CW. Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. Cytokine 1991;3:189-194.
31. Grucza R, Pekkarinen H, Titov EK, Kononoff A, Hanninen O. Influence of the menstrual cycle and oral contraceptives on thermoregulatory responses to exercise in young women. Eur J Appl Physiol 1993;67:279-285.
32. Henry JP. Biological basis of the stress response. N I P S 1993;8:69-73.
33. Hoffman JR, Maresh CM, Armstrong LE, Gabaree CLV, Bergeron, MF, Kenefick RW, Castellani JW, Ahlquist LE, Ward A. Effects of hydration status before and during mild exercise at elevated temperature on plasma testosterone, cortisol and catecholamines. Eur J Appl Physiol 1994;69:294-300.
34. Ho PC, Tang GWK, Lawton JWM. Lymphocyte subsets in patients with oestrogen deficiency. J Reprod Immunol 1991;20:85-91.
35. Hubbard RW, Armstrong LE. The heat illnesses: biochemical, ultrastructural, and fluid-electrolyte considerations. In: Human Performance Physiology and Environmental Medicine at Terrestrial Extremes. Pandolf KB, Sawka MN, Gonzalez RR (ed.). Indianapolis: Benchmark Press, 1988, pp. 305-359.
36. Hubbard RW, Gaffin SL, Squire DL. Heat-related illnesses. In: Management of Wilderness and Environmental Emergencies, 3rd edition. Auerbach PS (ed.). St. Louis: Mosby Year Book 1995;8:167-211.
37. Hubbard RW, Matthew WT, Thomas G, Sandick B. Bright Star 83 After Action Report. Natick, MA: U.S. Army Research Institute of Environmental Medicine, Heat Research Division; 1983.
38. Kaunitz AM. Long-acting injectable contraception with depot medroxyprogesterone acetate. Am J Obstet Gynecol 1994;170:1543-1549.
39. Krueger JM, Obal F, Opp M, Toth L, Johannsen L, Cady AB. Somnogenic cytokines and models concerning their effects on sleep. Yale J Biol Med 1990;63:157-172.
40. Marshall J. Thermal changes in the normal menstrual cycle. Brit Med J 1963;vol:102-104.
41. Mathur S, Mathur RS, Goust JM, Williamson HO, Fudenber HH. Cyclic variations in white cell subpopulations in the human menstrual cycle: correlations with progesterone and estradiol. Clin Immunol Immunopath 1979;13:246-253.
42. Novotny EA, Raveche ES, Sharow S, Ottinger M, Steinberg AD. Analysis of thymocyte subpopulations following treatment with sex hormones. Clin Immunol Immunopath 1983;28:205-217.
43. Parillo JE, Parker MM, Natanson C, Suffredini AF, Danner RL, Cunnion RE, Ognibene FP. Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. Ann Intern Med 1990;113:227-242.
44. Parker JL, Adams RH. Contractile dysfunction of atrial myocardium from endotoxin-shocked guinea pigs. Am J Physiol 1981;240:H954-H962.
45. Pivarnik JM, Marichal CJ, Spillman T, Morrow JR. Menstrual cycle phase affects temperature regulation during endurance exercise. J Appl Physiol 1992;72(2):543-548.
46. Prior JC, McKay DW, Vigna YM, Barr SI. Medroxyprogesterone increases basal temperature: a placebo-controlled crossover trial in postmenopausal women. Fertility & Sterility 1995;63:6.
47. Robert R, Chapelain B, Neliat G. Different effects of interleukin-1 on reactivity of arterial vessels isolated from various vascular beds in the rabbit. Circ Shock 1993;40:139-143.
48. Rowell LB. Human Circulation. Regulation During Physical Stress. New York: Oxford University Press, 1986, pp. 374-384.
49. Rowell LB, Blackmon JR, Martin RH, Mazzarella JA, Bruce RA. Hepatic clearance of indocyanine green in man under thermal and exercise stress. J Appl Physiol 1965;20:384-394.
50. Rowell LB, Marx J, Bruce RA, Conn RD, Kusumi F. Reductions in cardiac output, central blood volume and stroke volume with thermal stress in normal men during exercise. J Clin Invest 1966;45:1801-1816.
51. Shapiro Y, Alkan M, Epstein Y, Newman F, Magazanik A. Increase in rat intestinal permeability to endotoxin during hyperthermia. Eur J Appl Physiol 1986;55:410-412.
52. Sherman C. Stress: How to help patients cope. Phys Sports Med 1994;22:66-72.
53. Shibolet S, Lancaster MC, Danon Y. Heat stroke: a review. Aviat Space Environ Med 1976;47:280-301.
54. Shoham S, Davenne D, Cady AB, Dinarello CA, Krueger JM. Recombinant tumor necrosis factor and interleukin 1 enhance slow-wave sleep. Am J Physiol 1987;253:R142.
55. Simon HB. The immunology of exercise. JAMA 1984;252:2735-2738.
56. Siri WE. The gross composition of the body. In: Advances in Biological and Medical Physics New York: Academic Press, Inc., 1956, vol 4, p.239-280

57. Smiley K, Johannessen K, Marsh K, Collins C. Surveying University of Arizona student's health. J Am College Hlth 1992;41:75-77.
58. Speroff L, Glass RH, Rase NG. Long-acting methods of contraception. In: Clinical Gynecol Endocrinol Infertil, 5th ed. Baltimore: Williams & Wilkins; 1994.
59. Steinhampf MP, Blackwell RE. Contraception. In: Carr BR, Blackwell RE, eds. Textbook of Reproductive Medicine. Norwalk, CT: Appleton & Lange; 1993.
60. Straube W, Briese V. Sex steroids and the immune system. Zentralblatt fur Gynakologie 1989;111(9):552-558.
61. Thomas MD, Thomas PJ. Surveying pregnancy and single parenthood. In: Rosenfeld P, Edwards JE, Thomas MD, eds. Improving Organizational Surveys. Newbury Park: Sage; 1993.
62. Weicker H, Werle E. Interactions between hormones and the immune system. Int J Sports Med 1991;12:530-537.
63. Wells CL, Stern JR, Hecht LH. Hematological changes following a marathon race in male and female runners. Eur J Appl Physiol 1982;48:41-49.
64. Young AJ, Sawka MN, Epstein Y, Decristofano B, Pandolf KB. Cooling different body surfaces during upper body and lower body exercise. J Appl Physiol 1987;63:1218-1223.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 23 Aug 01

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the technical reports listed at enclosure. Request the limited distribution statement for these reports be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Reports to be Downgraded to Unlimited Distribution

ADB241560	ADB253628	ADB249654	ADB263448
ADB251657	ADB257757	ADB264967	ADB245021
ADB263525	ADB264736	ADB247697	ADB264544
ADB222448	ADB255427	ADB263453	ADB254454
ADB234468	ADB264757	ADB243646	
ADB249596	ADB232924	ADB263428	
ADB263270	ADB232927	ADB240500	
ADB231841	ADB245382	ADB253090	
ADB239007	ADB258158	ADB265236	
ADB263737	ADB264506	ADB264610	
ADB239263	ADB243027	ADB251613	
ADB251995	ADB233334	ADB237451	
ADB233106	ADB242926	ADB249671	
ADB262619	ADB262637	ADB262475	
ADB233111	ADB251649	ADB264579	
ADB240497	ADB264549	ADB244768	
ADB257618	ADB248354	ADB258553	
ADB240496	ADB258768	ADB244278	
ADB233747	ADB247842	ADB257305	
ADB240160	ADB264611	ADB245442	
ADB258646	ADB244931	ADB256780	
ADB264626	ADB263444	ADB264797	